TYROSINE HYDROXYLASE HAPLOINSUFFICIENCY PREVENTS AGE-ASSOCIATED ARTERIAL PRESSURE ELEVATION AND INCREASES HALF-LIFE IN MICE.

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**Running head:** Arterial pressure benefits in heterozygous TH mice.

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Abstract

Catecholamines are essential for the maintenance of physiological homeostasis under basal and stress conditions. We aim to determine the impact of deletion of a single allele of the tyrosine hydroxylase (Th) gene might have on aging arterial pressure and life-span. We found that Th haploinsufficiency prevents age associated increase of arterial pressure (AP) in mature adult mice, and it results in the extension of the half-life of TH-heterozygous (HET) mice respect to their wild-type (WT) littermates. Heart performance was similar in both genotypes. To further investigate the lack of increase in AP with age in TH-HET mice, we measured the AP response to intra-peritoneal administration of substances involved in AP regulation. The response to acetylcholine and the basal sympathetic tone were similar in both genotypes, while norepinephrine had a greater pressor effect in TH-HET mice, which correlated with altered adrenoreceptor expression in blood vessels and the heart. Furthermore, sympatho-adrenomedular response to stress was attenuated in TH-HET mice. Plasma catecholamine levels and urine glucose increased markedly in WT but not in TH-HET mice after stress. Our results showed that TH-HET mice are resistant to age-associated hypertension, present a reduction in the sympathetic response to stress and display an extended half-life.

Keywords (3-5): Catecholamines, tyrosine hydroxylase, hypertension, stress, life-span
1. Introduction

Hypertension is a major risk factor for ischemic heart disease, stroke and heart failure. Even moderate elevation of blood pressure can have a significant impact on health outcomes [1]. Moreover, the physiological increase in arterial pressure (AP) throughout life frequently manifests as progressive hypertension after midlife. Despite the importance of hypertension as a cause of cardiovascular and renal disease, its pathogenesis remains poorly understood in many cases. A variety of pathophysiological age-associated changes have been proposed for the development of age-dependent hypertension [reviewed in [2]], including structural changes in central arteries resulting in loss of the visco-elastic properties of conduit vessels [3,4,5,6]; decline of renal function [7,8]; local activation of the renin-angiotensin-aldosterone system [9] and sympathetic nervous system overactivity [10,11,12,13,14,15]. As many chronic disorders, age-associated hypertension is likely a multifactorial process in which the relative importance of the individual aspects remains to be determined.

Deregulated sympathetic activity has been involved in the development of other types of hypertension such as essential and secondary hypertension [16,17,18,19] and in various experimental models of hypertension [20,21,22,23,24,25]. However, the contribution of altered catecholaminergic homeostasis to age-associated hypertension is not well established.

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamines biosynthesis. TH catalyzes the conversion of the amino acid L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), which is sequentially converted into dopamine, norepinephrine (NE) and epinephrine. In humans, inactivating $Th$ mutations have severe motor and psychological consequences [26]. Moreover, allelic variations of $Th$ in humans correlate with changes in AP [27,28,29,30] and spontaneously hypertensive rats exhibit increased $Th$ expression [22].

In the present study, we addressed whether the loss of a single $Th$ allele does impact on basal hemodynamic arterial pressure homeostasis with age. This experimental model is closer to $Th$ genetic variations found in humans than the $Th$ null mouse since the later presents sever embryonic developmental alteration incompatible with life [31,32].
2. Materials and Methods

2.1. Animals

All procedures were approved by the Committee for Animal Care and Use of the University of Salamanca and complied with the Guide for the Care and Use of Laboratory Animals. As inactivation of both alleles in mice results in mid-gestational lethality: about 90% of mutant embryos die between embryonic days 11.5 and 15.5, apparently of cardiovascular failure [32], we have used tyrosine hydroxylase mice with only one allele targeted (Th-heterozygous: TH-HET). Generation of TH-HET mice on a C57BL/6 background has been previously described [32]. TH-HET mice were healthy and normal with no signs of any associated lesions. The growth rates of these animals were indistinguishable from those of WT animals, and normal reproduction was observed. Studies were performed in male TH-HET and their WT littermates. The number of animals in individual experiments ranged from 5 to 11. The specific number used for each study is referred in the corresponding Figure Legend. The animals were euthanized by cervical dislocation without previous anesthesia.

For basal catecholamine metabolite measurement, mice were placed in metabolic cages with water and food ad libitum. After 2 days of adaptation, 24 hour urine was collected in a collector tube with 100 µL of 1M HCl and samples were measured by high performance liquid chromatography in an external laboratory (Reference Laboratory, Barcelona, Spain).

2.2. Stress procedures

Restraint stress was performed in 50 mL plastic tubes. Ventilation was provided via several holes in the upper walls of the tube. Mice were restrained for 30 minutes. Blood sampling for plasma catecholamine measurements was performed at least 4 hours prior to application of restraint stress and immediately after the 30 minutes restraint stress. Mice were also subjected to stress by 1 minute of forced swimming. Pre- and post-stress blood samples were obtained by submandibular venipuncture.
For plasma separation, blood was collected in tubes containing EDTA (1 mM) and centrifuged at 1600 g for 15 minutes at 4°C.

Dopamine, NE and epinephrine plasma levels were quantified in plasma before and after restraint stress by ELISA (3-CAT Plasma ELISA high Sensitive, BA E-4600, Labor Diagnostika Nord, Nordhorn, Germany) following the supplier’s instructions.

Glycemia levels before and after forced swimming stress were measured by Glucometer Elite XL (Bayer Corporation, Elkhart, IN, USA).

2.3. Measurement of AP

Radiotelemetric AP measurement was performed via a catheter implanted into a carotid artery of the mouse, which in turn was attached to a combination pressure transducer, transmitter and battery, all encapsulated in an implantable microminiaturized electronic monitor (PA-C20, Data Sciences International (DSI); St. Paul, MN, USA). The devices were implanted as previously described [21,33]. At least 3 days after recovery, the cage housing the mouse was placed over a radio receiver and basal systolic and diastolic arterial pressure and heart rate were measured daily in each animal between 10 am and 2 pm for at least 10 days, to ensure that stable pressures were recorded. Data were digitally recorded on a computer and analyzed using the software provided by Data Sciences. To study the acute effects of the substances administered, animals implanted with transmitters were placed in the recording cage and after 1 hour of adaptation, basal systolic and diastolic arterial pressure and heart rate were recorded for 5 minutes. The mice were then injected intraperitoneally with the different substances to be tested (acetylcholine, 1 μg/kg; angiotensin II, 0.8 mg/kg; prazosin, 5 mg/kg; atenolol, 5 mg/kg; NE, 1 mg/kg) in 0.1 ml isotonic NaCl. Animals were then returned to the recording cages and arterial pressure and heart rate were continuously recorded for 30 minutes. Reserpine (2 mg/kg) was administrated daily for 3 days and AP was measured on the third day of treatment. The data shown are the average of the maximal hypertensive or hypotensive responses.

AP was also measured using tail-cuff equipment adapted for mice (Letica, Barcelona Spain) [33]. To minimize stress-induced fluctuations, mice were trained by measuring AP daily for more than 10
days between 9 and 12 am. Before measurement, the mice were placed for 10-20 minutes on a heating pad set at 28°C to facilitate detection of tail artery pulsations and to achieve a steady pulse level. The AP was recorded when at least 5 consecutive measurements provided similar values.

2.4. Mouse echocardiography

Transthoracic echocardiography was performed using a Vivid 7 (GE Medical Systems) cardiac ultrasound machine equipped with a 10-14-MHz iL3L transducer (GE Medical Systems), operated by a single experienced operator who was blinded to the mice genotype, as previously described [21]. The mice were lightly sedated by intraperitoneal administration of 45 mg/kg ketamine and maintained on a heated platform. Heart rates are generally maintained at more than 500 beats per minute with this regime. Each mouse was gently held in the palm of the hand by securing the skin of the dorsal neck with the thumb and index finger, securing the tail between the handler’s last two fingers. While in the supine position the chest hair was removed and pre-warmed hypoallergenic gel was applied to improve the ultrasonic penetration and the image quality. Care was taken to minimize excessive pressure of the transducer on the chest wall and to avoid reflex bradycardia. Measurement of chamber dimensions and cardiac function was performed offline (EchoPac Software, GE Medical Systems). Structural and functional heart parameters were calculated as previously described [21] and all echocardiographic parameters were averaged from at least three cardiac cycles.

2.5. RNA isolation and standard and quantitative RT-PCR

Total RNA from dissected tissues was isolated with the Trizol reagent (ThermoFisher Scientific, Waltham, MA USA) according to standardized provider information, and 2.5 μg of RNA was typically reverse transcribed (RT) with the Superscript III Kit and random primers (all from ThermoFisher). Quantitative PCR (qPCR) was performed with the ABI Prism 7900HT Sequence Detection System (ThermoFisher) using TaqMan Universal PCR Master Mix, No-AmpErase UNG (ThermoFisher), and probes from the Universal Probe Library (URL, Roche Applied Science) were used for detection. Primer sequences and the respective URL probes are listed in Table 1.
2.6. Statistical analysis

Data were analyzed with IBM SPSS Statistics for Windows, version 19.0 (IBM Corp, Armonk, NY) and GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA). The number of experiments performed is indicated in the figure legends. Thus, for non-paired data, Mann-Whitney U test was used to compare quantitative with qualitative variables of 2 categories and Kruskal–Wallis h test for qualitative variables of >2 categories. In the same way, Wilcoxon and Friedman test were used for paired data. The data are expressed as the mean ± SEM. A p value of ≤ 0.05 was considered statistically significant.

The life-span data were represented by the Kaplan Meier method for 11 WT and 5 TH-HET, without excluding censored mice, and the median life-span was calculated from these curves. The differences between TH-HET and WT mice longevity were tested using the Log-Rank method.
3. Results

3.1. TH heterozygous mice do not increase arterial pressure with age

A positive association between Th gene polymorphisms and essential hypertension has been previously described in humans [28,29]. We thus compared basal arterial pressure in TH-HET mice and WT littermates. In young adults (2-3 months of age), we observed no differences between WT and TH-HET mice for AP values obtained by tail-cuff (Fig. 1A). As basal AP increases throughout life in mice [34,35] and humans [36], we analyzed AP in mature adult (5-9 months of age) animals. In agreement with previous reports [34,35], we observed an increase in AP in mature versus young adult WT mice. By contrast, the AP of TH-HET mice did not increase with age (Fig. 1A). These differences were maintained in aged mice (24-months) (Supplementary Fig. 1). As a consequence, the AP of mature TH-HET mice was on average 20 mmHg lower than that of age-matched WT littermates. Moreover, the TH-HET mice presented longer half-life than the WT (984 days vs 834 days) although we did not observe differences in the maximum lifespan (Fig. 1B).

To determine whether the hemodynamic differences observed in mature TH-HET versus WT mice were associated with alterations in heart function and/or architecture, we performed echocardiography recordings in mature mice. The general heart architecture of both genotypes was very similar, with the exception of a significantly greater interventricular septum width and left ventricular mass in TH-HET mice (Table 2). Despite these differences, cardiac performance was similar in both genotypes, with normal ejection fraction, stroke volume and cardiac output (Table 2). Heart rate in the TH-HET mice was also comparable to that in WT mice (Table 2). Overall, these results indicate that the single cardiac structural difference observed in the TH-HET has no major impact on cardiac performance and is therefore unlikely to influence basal AP.

3.2. Characterization of the hemodynamic phenotype of Th heterozygous mice

To further characterize the hemodynamic differences between mature WT and TH-HET mice, we examined the effects of hypertensive and hypotensive agents on AP. Radiotelemetry was used to avoid
the possible confounding effects of movement restriction and to assess the acute effects of the compounds tested. In agreement with the tail-cuff recordings (Fig. 1), AP values, as measured by radiotelemetry, were significantly lower (an average of 20 mmHg) in mature TH-HET mice versus wild type littermates (Fig. 2A), maintaining similar heart rate (Fig. 2B).

The arterial endothelium is a major regulator of vascular homeostasis and hence of AP, and many models of hypertension are characterized by impaired endothelium-dependent vasorelaxation [37]. We thus examined the effect of the endothelium-dependent vasodilator acetylcholine. Similar decreases in AP were observed in WT and TH-HET mice after intraperitoneal acetylcholine administration (Fig. 2C) although the TH-HET mice showed a significant delay in reaching the maximal decrease (Supplementary Table 1). Angiotensin II, in turn, increased systolic AP in WT mice (by ~70 mmHg), while the TH-mice showed a trend to a lesser hypertensive response (~40 mmHg) although it did not reach statistical significance (Fig. 2E). No differences in the heart rate were observed after acetylcholine or angiotensin II treatments in either WT or TH-HET mice (Fig. 2D, 2F).

To compare the contribution of adrenoreceptors to the maintenance of resting AP in mature WT and TH-HET mice, we analyzed AP after injection of adrenergic receptor blockers. Prazosin, an α1-adrenergic antagonist, produced a comparable decrease in AP in both genotypes (Fig. 3A) although the maximal hypotensive response was obtained at a significantly later time (Supplementary Table 1). No changes in the heart rate (Fig. 3B) upon prazosin administration were observed in either WT or TH-HET mice. Similarly, no significant differences were observed between genotypes in the hypotensive effect of atenolol, a β1-adrenergic antagonist (Fig. 3C). Atenolol also induced a similar decrease in heart rate in both genotypes, confirming its β1-adrenergic blocking effect (Fig. 3D).

To further evaluate the adrenergic response of the TH-HET mice, mice were treated with reserpine prior to the administration of NE, a non-selective adrenergic receptor agonist. Reserpine which depletes catecholamines in peripheral sympathetic nerve endings, provoked similar decreases in AP in WT and TH-HET mice (Fig. 3E). However, subsequent administration of NE to catecholamine-depleted mice induced a significantly greater increase in AP in TH-HET than in WT mice (Fig. 3E).
3.3. Adrenergic receptor expression is altered in tissue-specific manner in TH-HET mice.

The increased responsiveness of mature TH-HET mice to exogenous NE administration suggested an elevated vascular catecholamine sensitivity in these mice. To determine whether changes in adrenoreceptor expression account for this increased sensitivity, we compared adrenoreceptor mRNA levels in mature TH-HET and WT mice in several tissues that have a major influence on overall hemodynamics. The expression of particular adrenoreceptor subtypes was altered in a tissue-specific manner (Fig. 4). In the splanchnic vessels, AR-α1D mRNA levels were significantly increased in TH-HET versus WT mice. In the renal artery, AR-α1A, AR-α1D and AR-β1 mRNA levels were increased in TH-HET versus WT mice. In the aorta, mRNA levels of AR-α1D were increased in TH-HET mice versus WT littermates, while those of AR-α2B and AR-β3 were decreased. In the cardiac analysis, separate analyses of the atrium and ventricle revealed chamber-specific changes in receptor mRNA expression. In the atrium, levels of AR-α1A, AR-α2C and AR-β3 mRNA were increased in TH-HET versus WT mice, while those of AR-α1D and AR-α2B were decreased. In the ventricle, levels of AR-α2A and AR-β3 mRNA were elevated in TH-HET mice as compared with WT littermates.

3.4. Plasma catecholamine levels did not rise after stress in TH-HET mice.

Although we observed a trend towards higher basal plasma levels of dopamine, NE and epinephrine in TH-HET versus WT mice these differences were not statistically significant due to the variability in TH-HET mice (Fig. 5A). In agreement with this observation, no significant differences in the urine catecholamine metabolite (homovanillic acid and vanillylmandelic acid) output were observed (Fig. 5B). After 30 minutes of restraint stress, plasma catecholamine levels were greatly increased in WT mice; dopamine by 9-fold and NE by 6-fold. More modest increases in epinephrine levels were observed, which failed to reach statistical significance. Surprisingly, plasma catecholamine levels did not increase after 30 minutes of restraint stress in TH-HET mice, as compared with basal levels (Fig. 5A). The TH-HET mice diminished response to stress was confirmed when we analyzed
the plasma glucose levels upon stress exposure. After 1 minute of forced swimming stress, the increment in the plasma glucose levels was significantly higher in the WT than in the TH-HET mice (Fig. 5C).
4. Discussion

Increasing age is considered a major risk factor for the development of cardiovascular disease [38], and hypertension represents a major challenge to cardiovascular health. One of the major findings of this study is the lack of age-associated AP increase observed in mature adult TH-HET mice as compared with WT littermates. Similar arterial pressure, assessed by tail cuff, was observed in both genotypes at 2-3 months of age. However, by 5-9 months of age, AP had increased in WT but not in TH-HET mice. In parallel we observed an increase in the half-life of the TH-HET mice with respect to their WT littermates. While the increase in AP with age has been previously reported in WT mice [34,35], the lack of AP increase in TH-HE mice prompted us to further characterize the impact of TH-haploinsufficiency on AP and catecholamine levels.

Echocardiography analysis demonstrated no significant differences in cardiac performance between mature TH-HET and WT adult mice, suggesting that the lower AP in the former group was not a consequence of reduced cardiac output, but more likely due to reduced peripheral resistance. The depressor response to acetylcholine was similar in both genotypes, indicating that higher endothelium-dependent vasorelaxation does not underlie the lower AP of TH-HET mice. Angiotensin II administration increased AP in both TH-HET and WT littermates, but was less effective in TH-HET mice. Angiotensin II exerts its hypertensive action by direct vasoconstriction of the vascular smooth muscle of blood vessels, and indirectly by stimulating the central and peripheral catecholamine release [39,40,41]. The tendency to reduced responsiveness of the TH-HET mice to angiotensin II may be due, at least in part, to a lower catecholaminergic surge upon angiotensin II stimulation. In agreement, we found that the catecholaminergic response to stress challenge was greatly diminished in TH-HET versus WT mice. The sympathetic nervous system plays a major role in hemodynamic adaptation to internal and environmental challenges [11,42]. We thus assessed the effect of specific adrenergic receptor blockade on arterial pressure in the mature adult TH-HET and WT mice. No significant differences were observed between genotypes in the hypotensive response to prazosin (α1-adrenergic antagonist) or to atenolol (β1-adrenergic antagonist). These results suggest a similar sympathetic tone in both genotypes in resting conditions. In addition, plasma catecholamine concentrations and urinary...
catecholamine metabolite excretion at rest did not differ significantly between genotypes. However, the TH-HET mice demonstrated an augmented pressor response to NE after catecholamine store depletion than the WT mice, suggesting an increased sensitivity to this neurotransmitter in the mutant mouse genotype. To determine whether changes in adrenoreceptor expression levels account for this enhanced noradrenergic response, we compared adrenoreceptor mRNA levels in several blood vessel types and in the heart in mature TH-HET and WT mice. In general, AR-α1 receptor mRNA levels were higher in blood vessels of TH-HET versus WT mice, although the expression profile of individual adrenoreceptor types differed for each vessel type. The adrenoreceptor expression profile observed in the arteries of TH-HET mice may, at least in part, account for the increased adrenergic pressor responsiveness, although it does not appear to underlie the lower basal AP observed in these mice. Additionally, the adrenoreceptor remodeling observed in the heart of mature adult TH-HET mice may account for the mild ventricular hypertrophy seen in these animals.

The mammalian response to stress is characterized by rapid activation of the sympathoadrenomedullary system and enhanced catecholamine release [reviewed in [42]]. Several studies have associated enhanced AP and augmented neuroendocrine and cardiovascular responses to acute stress [43,44,45,46]. Based on the observed similarities between genotypes in resting sympathetic tone (as indicated by the comparable responses to α- and β-adrenergic antagonists), we hypothesized that a reduced catecholaminergic response to stress may account for the lower AP seen in mature TH-HET compared with WT mice. In agreement, plasma levels of dopamine and NE were greatly increased after restraint stress in WT but not in TH-HET mice. Similarly, hyperglycemia after forced swimming, a hallmark of catecholamine metabolic response to stress, was lower in the TH-HET than in WT mice. These results suggest that the sympatho-adrenomedullary burst of catecholamine release after acute stress is abrogated in TH-HET mice. The mass discharge of catecholamines upon stress stimulation is due not only to release from the intracellular stores but also to a rapid increase in catecholamine synthesis [42]. Moreover, the enzymatic activity of TH (the limiting-step in catecholamine biosynthesis) is notably reduced in TH-HET compared with WT mice [47]. The present findings thus suggest that the decreased TH activity of monoallelic TH mice together with reduced catecholamine
reuptake and/or degradation are sufficient to maintain basal catecholamine levels [47] and the present study] but not to support the high demands of stress challenge or angiotensin II stimulation. While the lower basal AP of \(Th\)-haploinsufficient mice is in accordance with the hypotension observed in dopamine \(\beta\)-hydroxylase (DBH)-deficient humans and mice [48,49], the basal sympathetic tone of TH-HET mice is unaltered, whereas that of DBH-deficient subjects is dramatically reduced. Our results demonstrate that the sympatho-adrenomedullar response to stress is buffered in TH-HET mice, an effect that may contribute to the lower AP observed in mature TH-HET mice as compared with WT littermates. While it is generally assumed that the long-term regulation of AP is determined by the sympathetic tone at rest, our results suggest that the level of sympathetic response after stress may contribute to long-term AP regulation in TH-HET mice. With the increasing speed of modern life, the role of stress factors in the pathogenesis of hypertension has received increasing attention [50]. A strong acute stress or a chronic stress state can strongly affect the neuroendocrine system function [42] and participate in the development of hypertension. Our results suggest that the ability to cope with stress, at least as indicated by lower sympatho-adrenomedullar response in male mice, has a major impact in the regulation of AP and life-span. Future studies may address how \(Th\)-haploinsufficiency affects the AP circadian rhythm and its relation to longevity. In addition, studies in female mice may reveal whether there are any sex-differences in the sympatho-adrenomedullar response, the long-term AP regulation and life-span.

Acknowledgments

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Table 1: Primers and URL probes for qRT-PCR.

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<td></td>
<td>TTCCTCAACACCACATGAGC (right)</td>
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<td>AR-α1A</td>
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Table 2. Echocardiography parameters

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<td>N</td>
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<tr>
<td>Age (months)</td>
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<tr>
<td>Body weight (g)</td>
<td>29.8 ± 2.7</td>
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<tr>
<td>Heart rate (BPM)</td>
<td>586 ± 22</td>
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<tr>
<td>IVS (mm)</td>
<td>0.92 ± 0.01</td>
<td>1.01 ± 0.03*</td>
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<tr>
<td>LVPW (mm)</td>
<td>0.84 ± 0.02</td>
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<tr>
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<td>LVESV (µL)</td>
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<tr>
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<tr>
<td>Ejection fraction (%)</td>
<td>84.6 ± 2.4</td>
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<td>RWth (%)</td>
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<td><strong>Left ventricular mass (mg)</strong></td>
<td><strong>69.1 ± 3.4</strong></td>
<td><strong>93.3 ± 9.01</strong>*</td>
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IVS, interventricular septum; LVPW, left ventricular posterior wall; LVDd and LVDs, left ventricular internal dimensions at diastole and systole; LVEDV and LVESV, left ventricular end-
diastolic and end-systolic volumes; RWth, relative wall thickness. Results represent the mean ± SEM of 8 WT and 9 TH-HET mice. * p ≤ 0.05
Figure legends

**Figure 1. Arterial pressure and lifespan in WT and TH-HET mice.** (A) Comparison of arterial pressure between young (2-3 months of age) and mature adult (5-9 months of age) WT and TH-HET littermates. Arterial pressure was measured by tail-cuff. Results represent the mean ± SEM of 8 WT and 9 TH-HET mice. (B) Lifespan and half-life of 11 WT and 5 TH-HET littermates raised under identical housing conditions. * p ≤ 0.05; **p ≤ 0.01.

**Figure 2. Arterial pressure response to acetylcholine and angiotensin II.** Arterial pressure and heart rate were measured by telemetry in mice of 5-9 months of age that were untreated (A, B) or injected with acetylcholine (C, D) or angiotensin II (E, F). SAP, systolic arterial pressure; DAP, diastolic arterial pressure in B; bpm, beats per minute. AP and heart rate values after treatment were expressed relative to their respective basal values. Results represent the mean ± SEM of 8 WT and 9 TH-HET mice. * p ≤ 0.05; **p ≤ 0.01.

**Figure 3. Arterial pressure response to adrenergic agonists and antagonists.** Arterial pressure and heart rate were measured by telemetry following treatment with (A, B) prazosin, (C, D) atenolol, (E) reserpine, and reserpine followed by norepinephrine (NE). Results are expressed relative to their respective basal values and represent the mean ± SEM of 8 WT and 9 TH-HET mice. * p ≤ 0.05

**Figure 4. Adrenergic receptor expression.** Adrenoreceptor RNA levels were measured by quantitative RT-PCR of RNA from the indicated mouse tissues and normalized to levels of 18S ribosomal RNA. Results represent the mean ± SEM of at least 6 WT and 8 TH-HET mice. * p ≤ 0.05; ** p ≤ 0.001; # non detected.

**Figure 5. Comparison of catecholamine, catecholamine metabolites and glucose levels.** (A) Plasma catecholamine (dopamine, norepinephrine and epinephrine) levels were measured at rest and after 30 minutes restraint stress (RS). Values for each individual (6 WT and 8 TH-HET mice) as well as the mean value for each group are plotted. (B) Dopamine metabolite (homovanillic acid) and epinephrine and norepinephrine metabolite (vanillylmandelic acid) were measured in the 24 hours-
collected urine of WT and TH-HET mice at rest. Results represent the mean ± SEM of at least 6 WT and 6 TH-HET mice. Plasma glucose levels were measured after 1 minute of force swimming stress. Results represent the mean ± SEM of at least 5 WT and 5 TH-HET mice. * p ≤ 0.05.
References


Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Highlights

- A lack of age-associated AP increase found in mature adult $Th$-heterozygous mice.
- The $Th$-heterozygous mice had an increased half-life.
- The catecholaminergic response to stress was diminished in the $Th$-heterozygous group.