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Original Paper

Global Overexpression of ET-1 Decreases Blood Pressure – A Systematic Review and Meta-Analysis of ET-1 Transgenic Mice

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Key Words
Endothelin-1 transgenic mice • Blood pressure • Kidney weight • Heart weight

Abstract
Background/Aims: ET-1 has independent effects on blood pressure regulation in vivo, it is involved in tubular water and salt excretion, promotes constriction of smooth muscle cells, modulates sympathetic nerve activity, and activates the liberation of nitric oxide. To determine the net effect of these partially counteracting mechanisms on blood pressure, a systematic meta-analysis was performed. ***Methods:*** Based on the principles of Cochrane systematic reviews, we searched in major literature databases - MEDLINE (PubMed), Embase, Google Scholar, and the China Biological Medicine Database (CBM-disc) - for articles relevant to the topic of the blood pressure phenotype of endothelin-1 transgenic (ET-1+/+) mice from January 1, 1988 to March 31, 2016. Review Manager Version 5.0 (Rev-Man 5.0) software was applied for statistical analysis. In total thirteen studies reported blood pressure data. ***Results:*** The meta-analysis of blood pressure data showed that homozygous ET-1 transgenic mice (ET-1+/+ mice) had a significantly lower blood pressure as compared to WT mice (mean difference: -2.57 mmHg, 95% CI: -4.98~ -0.16, $P = 0.04$), with minimal heterogeneity ($P = 0.86$). A subgroup analysis of mice older than 6 months revealed that the blood pressure difference between ET-1+/+ mice and WT mice was even more pronounced (mean difference: -6.19 mmHg, 95% CI: -10.76~ -1.62, $P = 0.008$), with minimal heterogeneity ($P = 0.91$). ***Conclusion:*** This meta-analysis provides robust evidence that global ET-1 overexpression in mice lowers blood pressure in an age-dependent manner. Older ET-1+/+ mice have a somewhat more pronounced reduction of blood pressure.

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Introduction

As early as in 1985 Hickey et al. [1] found an endothelium-derived constricting factor by analyzing the supernatant of bovine aortic endothelial cells. This factor seemed to be a peptide, since trypsin abolished the observed activity [1]. The molecular structure of this endothelium-derived constricting factor was identified 3 years later by Yanagisawa et al. [2] He named this factor endothelin, because he isolated it from the supernatant of porcine aortic endothelial cells. This peptide was a very strong – probably the strongest so far described – vasoconstrictor. It produces powerful, very long-lasting constrictions of a range of mammalian blood vessels in vitro injection, including human arteries and veins. It also causes long-lasting elevation of blood pressure when injected into rodents [2]. These observations led to the hypothesis that this peptide – nowadays called endothelin-1 (ET-1), since there are two further endothelin isoforms that were discovered shortly after the initial discovery of ET-1 – plays a pivotal role in the pathogenesis of hypertension [3]. It was thus a completely unexpected finding that ET-1 transgenic mice do not develop hypertension. This was first shown by our group in Berlin, Germany [4]. ET-2 overexpressing rats likewise do not develop hypertension [5]. Both rat and mouse ET overexpression models develop renal intestinal fibrosis and glomerulosclerosis in a blood pressure independent manner [4-8]. When going to the original publications, it was always noted that numerically the blood pressure was even somewhat lower in ET-1 transgenic mice as compared to their WT control counterparts. The difference was numerically small but always detectable. These studies were most likely underpowered to detect a potential small blood pressure lowering effect of global ET-1 gene overexpression. The aim of the current study therefore was to perform a systematic review and meta-analysis of all published studies on global ET-1 overexpression in the past two decades. We likewise analyzed data on heart and kidney weights as well as data on urinary protein excretion and heart rate derived from these publications.

Materials and Methods

Search strategy

Two authors screened published articles in MEDLINE (PubMed), Embase, Google Scholar, and CBM-disc, for relevant articles comparing cardiovascular and renal effects of ET-1 genetic overexpression with corresponding controls in murine models in the period of time from January 1, 1988 to March 31, 2016. The searching keywords were “endothelin-1 transgenic mice”, “endothelin-1 blood pressure or hypertension”, and “endothelin-1 overexpressing, kidney or cardiac”.

Inclusion and exclusion criteria

In order to be included in the present meta-analysis, each trial must have clearly described at least one of the following parameters: systolic blood pressure (SBP), heart rate (HR), heart/body weight (HW/BW) ratio, urinary protein excretion/24h, and kidney/body weight (KW/BW) ratio. Only articles reporting on male mice were included, since there were too few studies analyzing female mice.

Data extraction and quality assessment

Two authors selected relevant articles, summarized data, and evaluated quality of included articles independently. We contacted the authors in the case if the information available from the original articles was unclear or incomplete. The trials’ quality were independently assessed by two review authors according to the 20-item checklist of the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines [9]. One score of trials’ quality in our meta-analysis equals to one item of the ARRIVE guidelines. The higher scores indicate higher quality of the study. The data on SBP, HR, heart/body weight ratio, urinary protein excretion/24h, and kidney/body weight ratio were extracted from the articles, which met the aforementioned criteria. Data were given as mean ± SD. In the cases, when the original data were given as means ± SEM, we calculated SD form SEM (using the formula $SD = SEM \times \sqrt{n}$, where n is the sample size for each group).

Table 1. Systolic blood pressure, heart rate, and heart / body weight ratio for WT and ET-1+/+ mice

First Author, Year	SBP (mmHg)		HR (bpm)		HW/BW ratio (%)	
	WT	ET-1+/+	WT	ET-1+/+	WT	ET-1+/+
Overexpression of Endothelin-1 in Whole Body						
Hocher, 1997						
3 months	85.9±4.2	88.9±9.3	-	-	6.0±0.4	5.1±0.4
14 months	96.0±6.1	86.9±14.6	-	-	5.0±0.5	6.7±0.5*
Theuring, 1998						
14 months	116±8	107±10	-	-	-	-
Schwarz, 2002						
12 months	98±8	93±9	-	-	5.0±0.5	6.7±0.5
Shindo, 2002						
8-10 weeks	121±3	120±6	674±82	660±37	-	-
Quaschning, 2003						
6 months	116±12.2	118±12.2	586±61	578±69	-	-
Hocher, 2004						
3 months	94.4±7.3	89.0±8.8	-	-	-	-
Quasching, 2007						
Age nnclear	94.4±24.4	95.7±10.2	-	-	-	-
Quasching, 2008						
Age unclear	117±22.6	108±26.2	-	-	-	-
Kalk, 2008						
13 months	118±22.6	109±15.8	426±37	432±47	-	-
Vignon-Zellweger, 2011						
9 months	94±46.5	93±41.2	621±87	645±60	4.8±1.5	5.6±2.6
Vignon-Zellweger, 2014						
3 months	90.97±16.62	91.01±10.07				
5 months	99.0±13.71	97.33±12.91	-	-	-	-
9 months	107.13±11.45	106.81±13.55				
Satwiko, 2015						
13-18 weeks	104.0±13.21	90.25±19.31	-	-	-	-
Tsuprykov, 2015						
3 months	91.0±16.6	91.0±10.1	-	-	-	-
9 months	119.2±14.4	109.7±22.6	-	-	-	-
Overexpression of Endothelin-1 in Endothelium						
Amiri, 2004						
10 weeks	128±9.8	135±9.8	-	-	-	-
Li, 2013						
14 weeks	100±10	111±18	659±44	710±60	-	-

WT, -wild type; ET-1+/+, -ET-1 transgenic; SBP, -systolic blood pressure.

Statistical analysis

Statistical analyses were conducted by Review Manager Version 5.0 (Rev-Man 5.0) software, designed by the Cochrane Collaboration. Results were expressed as the mean difference (MD) and 95% confidence intervals (CI) and were obtained based on fixed-effect or random-effect model of random-effect model of inverse variance (IV). Heterogeneity was assessed by P value and I^2. In the cases with minimal heterogeneity ($P \geq 0.1$, $I^2 \leq 50\%$), we used the fixed-effect model of IV; in the cases with distinguished heterogeneity ($P < 0.1$, $I^2 > 50\%$) we used the random-effect model of IV. Among 13 included articles [4, 6, 10-22], the variation of the age was high - from 8 weeks until 14 months. To decrease the influence of mice age on the final outcome, we divided the data into the following subgroups: mice aged 0-6 months and mice aged older than 6 months. In rare cases, when the animal age was not clear, we established one more subgroup called "age unclear". In tests for overall effect, the $P \leq 0.05$ was considered statistically significant.

Results

Basic information

Based on the principle of Cochrane systematic reviews, and the inclusion and exclusion criteria of the present meta-analysis, 15 articles have finally been included [4, 6, 10-22].

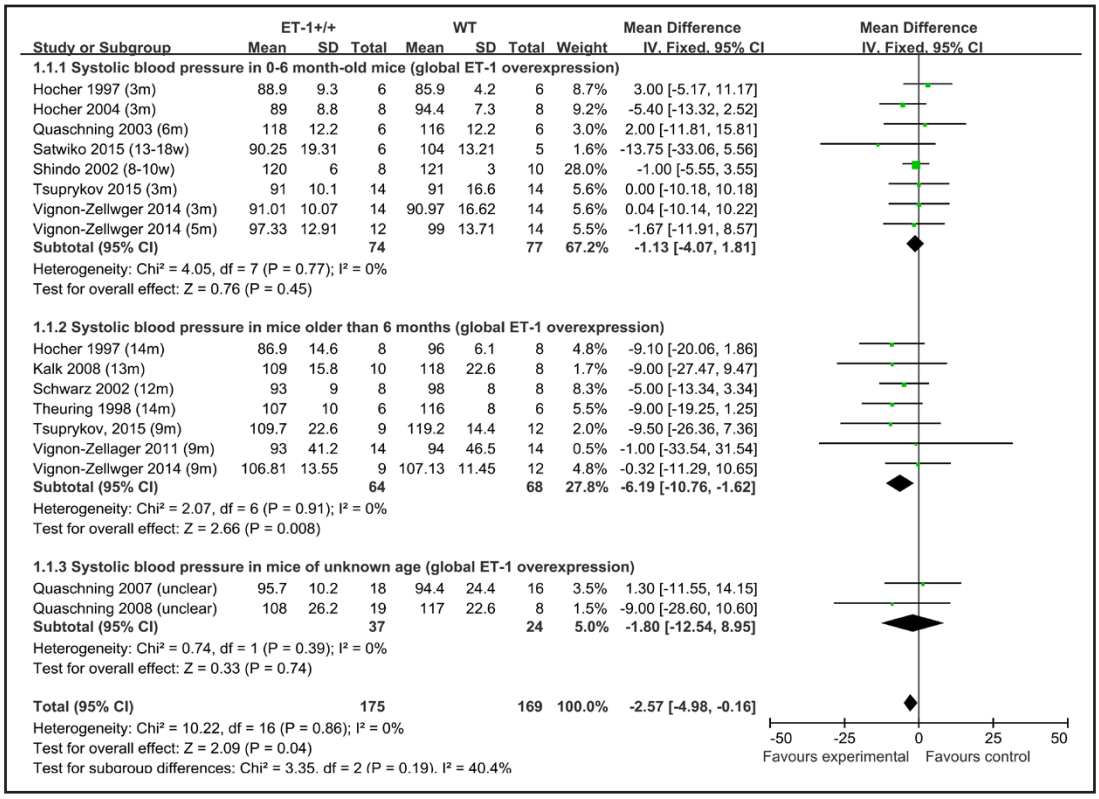

Fig. 1. Forest blots depicting systolic blood pressure in mice with global ET-1 overexpression and WT mice.

For the ET-1+/+ mice, there were thirteen studies with ET-1 global overexpression(whole-body overexpression) [4, 6, 10-20], and two studies with endothelium-restricted ET-1 overexpression [21, 22].

Blood pressure

Thirteen studies described blood pressure data in ET-1+/+ mice in comparison to wild-type mice [4, 6, 10-22], (table 1). ET-1+/+ mice had a significantly lower blood pressure (mean difference: -2.57 mmHg, 95% CI: -4.98∼-0.16, $P = 0.04$), with minimal heterogeneity ($P = 0.86$, $I^2 = 0\%$). A subgroup analysis of mice older than 6 months revealed that the blood pressure difference between ET-1+/+ mice and WT mice was even more pronounced (mean difference: -6.19 mmHg, 95% CI: -10.76∼-1.62, $P = 0.008$), with minimal heterogeneity ($P = 0.91$, $I^2 = 0\%$). (figure 1)

Heart rate (global ET-1 overexpression)

Four studies reported heart rate data [6, 12, 16, 17] (table 1). The overall effect was neutral: no differences concerning heart rate between WT and ET-1+/+ mice were detectable ($P = 0.76$), with minimal heterogeneity ($P = 0.80$, $I^2 = 0\%$). Subgroup analysis of 0-6 month-old mice and mice older than 6 months likewise showed no difference between ET-1+/+ mice and WT mice (all $P > 0.05$) with regards to heart rate.

Heart weight/body weight (HW/BW) ratio (global ET-1 overexpression)

Three articles described HW/BW ratios [4, 11, 17] (table 1). Overall, the HW/BW ratio showed no difference between ET-1+/+ and WT mice ($P = 0.28$), with distinguished heterogeneity ($P < 0.0001$, $I^2 = 96\%$). Interestingly, a subgroup analysis of 0-6 month-old mice detected that young ET-1+/+ mice showed significantly lower HW/BW ratio than WT mice (mean

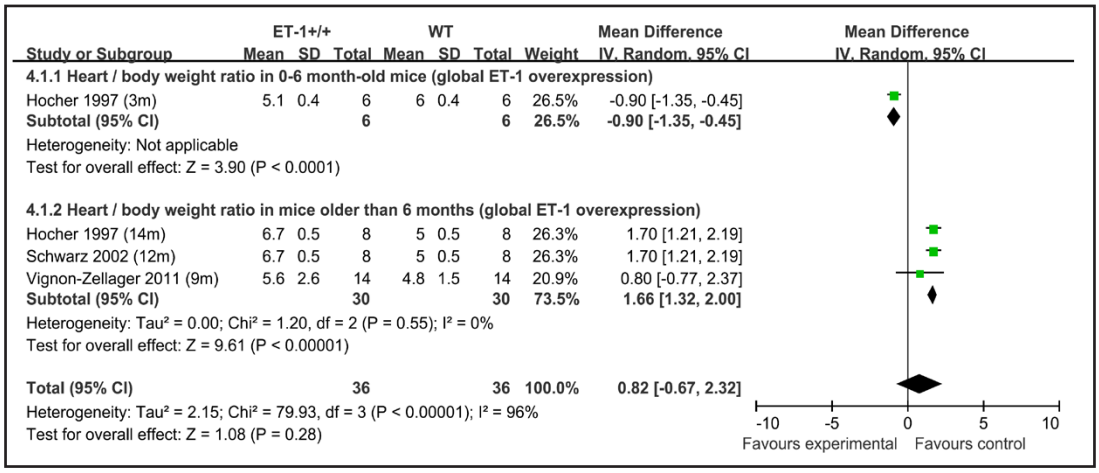

Fig. 2. Forest blots depicting heart/body weight ratio in mice with global ET-1 overexpression and in WT mice.

difference: -0.90%, 95% CI: -1.35~-0.45, $P < 0.0001$). On the other hand, the subgroup analysis of mice older than 6 months, revealed that ET-1+/+ mice had a significantly higher HW/BW ratio than WT mice (mean difference: +1.66%, 95% CI: 1.32~2.00, $P < 0.0001$), with minimal heterogeneity ($P = 0.55$, $I^2 = 0\%$; for more details, see figure 2).

Table 2. Urinary protein excretion/24h, and kidney/body weight ratio for WT and ET-1+/+ mice

First Author, Year	Proteinuria (mg/24h)		KW/BW ratio (%)	
	WT	ET-1+/+	WT	ET-1+/+
Hocher, 1997				
3 months	-	-	7.0±0.34	7.5±0.84
14 months	2.93±0.39	2.51±0.50	7.5±0.8	8.8±1.0
Schwarz, 2002				
12 months	-	-	7.5±0.8	8.8±1.0
Shindo, 2002				
8-10 weeks	26.9±5.4	32.9±5.1	-	-
Hocher, 2004				
3 months	-	-	7.1±0.32	6.6±0.59
Tsuprykov, 2015				
9 months	1.98±0.45	3.39±1.25	12.20±1.59	14.10±1.38

Urinary protein excretion/24h (global ET-1 overexpression)

Three studies described urinary protein excretion/24h [4, 6, 20] (table 2). In total, no difference between ET-1+/+ and WT mice with respect to urinary protein excretion/24h was observed ($P = 0.26$), with distinguished heterogeneity ($P < 0.0001$, $I^2 = 90\%$;for more details, see figure 3).

Kidney weight/body weight (KW/BW) ratio (global ET-1 overexpression)

Four studies provided KW/BW ratio data (table 2). In general, there was a trend towards higher KW/BW ratio in ET-1+/+ mice in comparison to WT mice [4, 11, 13, 20] (mean difference: 0.82%, 95% CI: -0.10~1.74), $P = 0.08$), with distinguished heterogeneity ($P < 0.0001$, $I^2 = 85\%$). Subgroup analysis of 0-6 month-old mice revealed no difference between ET-1+/+ and WT mice ($P = 0.94$), with distinguished heterogeneity ($P = 0.02$, $I^2 = 81\%$), whereas ET-1+/+ mice older than 6 months had a significantly higher KW/BW ratio than WT mice (mean difference: 1.42%, 95% CI: 0.85~1.98, $P < 0.0001$), with minimal heterogeneity ($P = 0.71$, $I^2 = 0\%$; for more details, see figure 4).

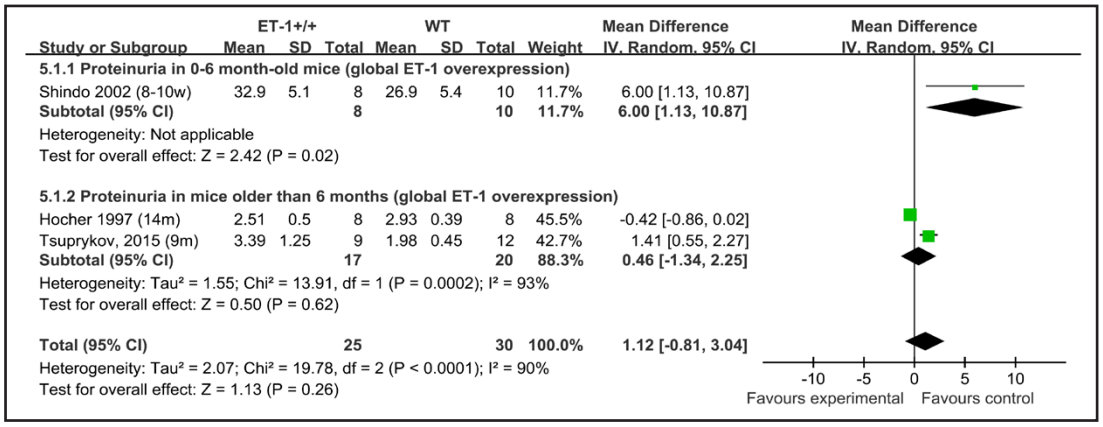

Fig. 3. Forest blots depicting urinary protein excretion/24h in mice with global ET-1 overexpression and in WT mice.

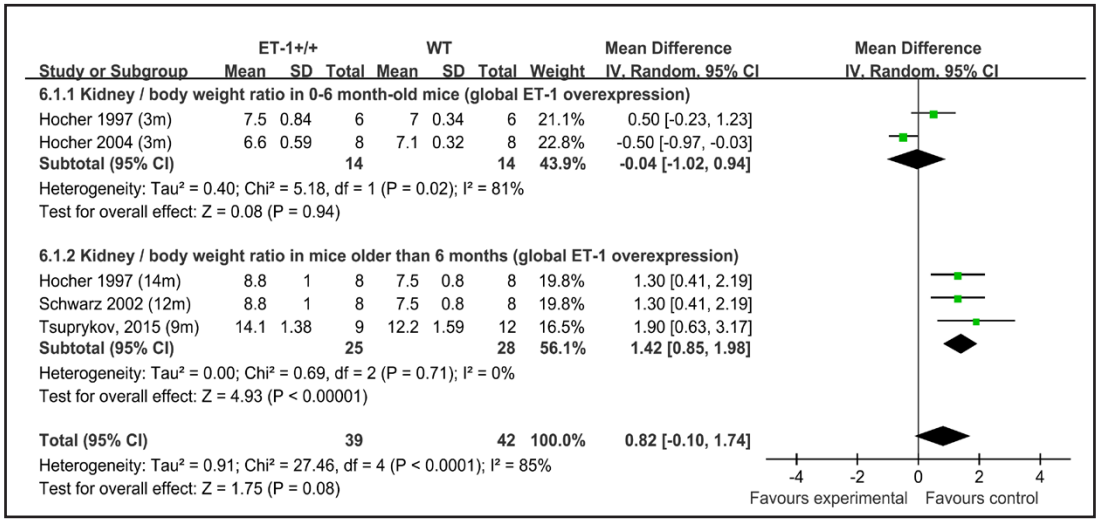

Fig. 4. Forest blots depicting kidney/body weight ratios mice with global ET-1 overexpression and in WT mice.

Discussion

This meta-analysis demonstrated that blood pressure is lower in ET-1+/+ mice as compared to WT mice. In particular, mice older than 6 months had lower blood pressure as compared to WT controls. Older ET-1+/+ mice also exhibited a higher kidney/body weight ratio. ET-1+/+ mice did not develop proteinuria. ET-1+/+ mice older than 6 months were also characterized by an increased heart/body weight ratio.

Blood pressure

Our study revealed that global ET-1 overexpression in mice lowers blood pressure in an age-dependent manner. Older ET-1+/+ mice have a somewhat more pronounced reduction of blood pressure when compared to WT mice of the same age (see table 1 for individual studies in the past two decades and figure 1 for the meta-analysis). This fits well with the observation that a global heterozygous ET-1 knockout in mice causes hypertension (the complete knockout in homozygous ET-1 knockout mice is lethal due to craniofacial

malformations [23]). This landmark study was recently confirmed by another independent study showing that a decrease in systemic ET-1 level of up to 35 % of that in the control mice causes hypertension [24]. Notably, in this model blood pressure could be normalized by epithelial sodium channel blockers, indicating that renal tubular sodium transport plays a key role in the pathogenesis of hypertension in these settings. ET-1 has numerous independent effects on blood pressure regulation in vivo, it is involved in tubular water and salt excretion, promotes constriction of smooth muscle cells, modulates sympathetic nerve activity, and activates the liberation of nitric oxide via an ETB receptor-mediated pathway from endothelial cells. The latter effect is responsible for the small short-lasting decrease of blood pressure following immediately after an injection of ET-1 [25, 26]. Animal models with cell type-specific overexpression or knockout of ET-1 are suitable experiment tools to dissect the different and sometimes opposite ET-1 effects on blood pressure in vivo [25, 26]. One classical example of a local cellular ET-1 knockout animal model demonstrating renal tubular effects of ET-1 involved in blood pressure regulation is collecting duct-specific ET-1 knockout mice (CDET-1KO). The inner medullary collecting ducts (CD) have the highest density of ETB receptors in the body and produce large amounts of ET-1 [25, 26]. Deletion of ET-1 from the CD resulted in a salt sensitive blood pressure phenotype. When fed a normal salt diet, CDET-1KO mice had significantly higher systolic blood pressure compared with their floxed control littermates [27]. These mice had sodium retention, and this could be ameliorated with amiloride or furosemide suggesting that the epithelial sodium channels (eNAC) are involved in this pathway. However, there is evidence that blood pressure is also controlled by other mechanisms than tubular water and salt retention [27] in CDET-1KO mice. In this model, blood pressure responds very well to treatment with angiotensin receptor blockers or mineralocorticoid receptor antagonists indicating that partially the hypertension in the CDET-1KO mouse is due to a relative renin-angiotensin-aldosterone system overactivity [28].

Another cell type-specific model is mice with endothelial cell-selective ET-1 overexpression. The ET-1 synthesized in the endothelial cells seems to activate directly vascular ET receptors (mainly ETA receptors on smooth muscle cells). Endothelial ET-1 overexpressing models develop an increased blood pressure [29, 30], whereas in another model endothelial-selective ET-1 overexpression does not results in a significant blood pressure elevation, although blood pressure in this model was numerically higher [21]. This is most likely a matter of power in this study, since the number of tested animals was most likely too low to detect significant differences in blood pressure levels. The analysis of both studies together with the statistical approach used in the present study (see "Materials and Methods" section) revealed that there was a strong trend ($p=0.06$, data not shown) for blood pressure elevation in endothelium-restricted ET-1 transgenic mice.

The apparently contradictory findings between the initial report by Yanagisawa et al. [2] describing ET-1 as a very potent vasoconstrictor and the subsequent evidence that this peptide does not elevate blood pressure in transgenic mice in vivo models [4, 6], might be due the fact that the experiments done by Yanagisawa et al. [2] tested basically just the effects of ET-1 on the ETA receptor in smooth muscle cells. Potential tubular effects of ET-1 are simply not detectable by in vivo single bolus injections of ET-1 nor by aortic ring experiments. In ET-1+/+ animal models, the measured blood pressure represents the integrated effects of ET-1 mediated blood pressure elevating mechanisms and ET-1 mediated blood pressure lowering mechanisms.

Taken together, this meta-analysis indicates that the net effect of global ET-1 overexpression causes a reduction of blood pressure. The partial (heterozygous) global knockout or decreased ET-1 mRNA expression – on the other hand – may cause an elevation of blood pressure.

Kidney weight and protein excretion

The increase of the kidney to body weight ratio (relative kidney weight) in ET-1+/+ mice is most likely due to the age-dependent development of small kidney cysts in these

mice [4, 6]. Urinary protein excretion was not increased in ET-1+/+ mice. On the other hand, ET-2 transgenic (ET-2+/+) rats develop mild proteinuria [5]. Expression analysis using in situ hybridization and receptor autoradiography revealed that glomerular transgene expression rates in ET-2+/+ rats are much higher as compared to ET-1+/+ mice [4, 5, 31]. It is of note, however, that even the relatively strong glomerular transgene expression in ET-2+/+ rats results just in mild proteinuria, whereas ET blockers are very effective in reducing proteinuria in experimental models of diabetic nephropathy [32-34] and also in humans with diabetic kidney disease [35-40]. Our meta-analysis indicates that ET-1 seems not to cause considerable damage to the protein filtering apparatus in the glomeruli (endothelial cells, glomerular basement membrane and podocytes) on its own without any further stimuli, but becomes very important in the pathogenesis of proteinuric kidney disease, if the molecular sieve is damaged primarily by other stimuli such as a long-lasting impairment of glycaemia (diabetes). Short term activation of the endothelaial ET-1 synthesis in endothelial ET-1 transgenic mice did not result in a renal phenotype [30]. This is consistent with findings in global ET-1 overexpressing mice, because the development of the renal phenotype in these mice was seen in a age dependent manner [4]. Three weeks are clearly too short for the development of an ET-1 indiced renal phenotype [4, 30]

Heart weight

The heart/body weight ratio (relative heart weight) in young mice seems to be decreased as compared to age-matched controls (figure 2). This statement, however, is just based on one study with just 6 animals in each group and hence needs to be considered as a preliminary result requiring independent confirmation. The heart/body weight ratio in older ET-1+/+ mice, on the other hand, is clearly increased as compared to control littermates of the same age (figure 2). This is remarkable, since blood pressure is clearly lower in ET-1+/+ mice in this age group indicating that there are obviously ET-1 mediated blood-pressure independent mechanisms increasing the heart weight. Vignon-Zellweger et al. [17] demonstrated a two-fold elevated cardiac ET-1 mRNA expression in ET-1+/+ mice compared to the WT counterparts. Brain natriuretic peptide concentrations were slightly but not significantly higher in ET-1+/+ mice. Heart catheter examination of these mice revealed no major differences between WT mice and ET-1+/+ mice with the exception of a significantly decreased time of left ventricular relaxation constant (tau) in ET-1+/+ mice. Myocyte size and cardiac collagen content were similar in WT and ET-1+/+ mice [17]. However, under conditions of a 10-fold increase of cardiomyocyte-derived ET-1 production, observed in a mouse model of cardiomyocyte-specific ET-1 overexpression, the cardiac phenotype was characterized by an increased expression of inflammatory cytokines and an inflammatory cardiomyopathy leading to heart failure and death [41]. Taken together, mild and most likely physiological up-regulation of cardiac ET-1 expression causes no considerable damage to the heart, whereas extremely elevated ET-1 levels result in the development of an inflammatory cardiomyopathy. These findings are consistent with findings in uremic and/or hypertensive rat models. In these rats it was shown that an ETA receptor blocker reduces the size of myocytes in a blood pressure independent manner [42, 43].

Study limitations

The data on ET-1+/+ mice are based just on several transgenic mouse lines. These mouse lines, however, were independently developed in a Germany group and a Japanese group. The phenotypic characterization – in particular blood pressure measurements - were conducted independently in four laboratories (two in Germany – Charité - Universitätsmedizin Berlin and the University of Würzburg – and two in Japan – the University of Tokyo and the University of Kobe). The findings were remarkable constant over the past two decades (see table 1). With the exception of blood pressure data (reported in 13 studies), data concerning heart weight, kidney weight, and 24 hour protein excretion were reported only in 3 – 5 studies. Thus the key finding of this meta-analysis – lower blood pressure in ET-1 transgenic mice

as compared to WT mice - is based on a robust data set, whereas data especially on urinary protein excretion are just based on a limited number of studies.

Conclusion

In conclusion, global ET-1 overexpression in mice did not cause proteinuria but led to a slight reduction of blood pressure. This fits very well with the observation that a decreased tissue concentration or a heterozygous knockout of the ET-1 gene is associated with an elevation of blood pressure [24]. Despite lower blood pressure, the heart/body weight ratio in older ET-1+/+ mice is clearly increased as compared to control littermates of the same age. Moreover, our study challenges the hypothesis that ET-1 plays a major role in the pathogenesis of human hypertension,

Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

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