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Translational value of mechanical and vasomotor properties of mouse isolated mesenteric resistance-sized arteries

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Abstract

Mice are increasingly used in vascular research for studying perturbations and responses to vasoactive agents in small artery preparations. Historically, small artery function has preferably been studied in rat isolated mesenteric resistance-sized arteries (MRA) using the wire myograph technique. Although different mouse arteries have been studied using the wire myograph no establishment of optimal settings has yet been performed. Therefore, the purposes of this study were firstly to establish the optimal settings for wire myograph studies of mouse MRA and compare them to those of rat MRA. Second, by surveying the literature, we aimed to evaluate the overall translatability of observed pharmacological vasomotor responses of mouse MRA to those obtained in rat MRA as well as corresponding and different arteries in terms of vessel size and species origin. Our results showed that the optimal conditions for maximal active force development in mouse MRA were not significantly different to those determined in rat MRA. Furthermore, we found that the observed concentration-dependent vasomotor responses of mouse MRA to noradrenaline, phenylephrine, angiotensin II, sarafotoxin 6c, 5-hydroxytryptamine, carbachol, sodium nitroprusside, and retigabine were generally similar to those described in rat MRA as well as arteries of different sizes and species origin. In summary, the results of this study provide a framework for evidence-based optimization of the isometric wire myograph setup to mouse MRA. Additionally, in terms of translational value, our study suggests that mouse MRA can be applied as a useful model for studying vascular reactivity.

Abbreviations

5-HT, 5-hydroxytryptamine; Ang II, angiotensin II; AWT, active wall tension; EDHF, endothelium-derived hyperpolarizing factor; IC, internal circumference; MRA, mesenteric resistance-sized arteries; NA, noradrenaline; NO, nitric oxide; PE, phenylephrine; PSS, physiological salt solution; PWT, passive wall tension; S6c, sarafotoxin 6c; SNP, sodium nitroprusside.

Introduction

Rodent animal models are commonly used in vascular research. Mouse models have become increasingly popular compared to rats due to several advantages such as cost-efficiency and a well-studied genome allowing genetic manipulation to mimic several human disease models (Young and Davisson 2011). In particular, the C57BL/6 mouse strain is widely used for studying vascular changes in various models of diabetes, hypertension, and vascular disease (Taherzadeh et al. 2010; Wang and Liao 2012). A common hallmark of these disorders is perturbation of small artery function (i.e., proximal resistance-sized vessels with internal diameters of 100–400 μm) (Mulvany and Aalkjaer 1990). Although investigations of isolated small artery function were revolutionized by the develop-

ment of the myograph technique decades ago (Mulvany and Halpern 1976), rat mesenteric resistance-sized arteries (MRA) have predominantly been used as a model in these investigations. MRA are a highly preferred vascular bed for functional studies of small arteries in part due to their dense innervation and significant role in blood pressure and tissue perfusion regulation (Furness and Marshall 1974). Furthermore, MRA are found in large quantities, and are relatively easily dissected out and mounted without significant tissue damage (Warshaw et al. 1979).

Wire myograph studies of isolated small arteries are initiated with a normalization procedure. The purpose of this procedure is to ensure optimal conditions for maximal active force production of the individual artery preparation before assessing pharmacological vasoreactivity. Empirical data obtained in rat MRA by Mulvany et al. have served as the gold standard for this important normalization procedure (Mulvany and Halpern 1977; Mulvany and Nyborg 1980). Although very similar in outward appearance, the mouse cannot be considered a small rat (Rats! [Editorial], 2010). Along these lines, it is not clear whether the optimal settings for normalization of rat MRA can be reflected in mouse MRA (van den Akker et al. 2010).

Vasomotor responses of mouse MRA to various vasoactive agents have previously been characterized using the wire myograph technique (Yamamoto and Koike 2001; Hedemann et al. 2004; Longo et al. 2005; Yeung et al. 2007; Takaki et al. 2008; Matsumoto et al. 2010; Harrington et al. 2011; Hassanain et al. 2013; Kleinbongard et al. 2013). However, to our knowledge, the overall translational value of the mechanical and vascular pharmacological profile of mouse MRA to other rodents, such as rat, and in particular to humans, has yet to be addressed.

Therefore, the purposes of this study were firstly to establish the optimal settings for the normalization procedure in mouse MRA and compare them to those of rat MRA. Second, by surveying the literature, we aimed to evaluate the translatability of observed pharmacological vasomotor responses of mouse MRA to those obtained in rat MRA as well as corresponding and different arteries in terms of vessel size and species origin. The vasoactive agents investigated were selected based on their differential involvement in controlling vascular tone of resistance-sized arteries. The list covers different physiological origins and actions involving various receptors, ion channels, and signaling pathways. Furthermore, both endothelium-dependent and endothelium-independent vasodilations were investigated.

Briefly, we found that the mechanical and pharmacological properties of mouse MRA were rather similar to those observed and described in rat MRA as well as arteries of different sizes and species origin with a few exceptions for responses to 5-HT and Ang II due to heterogeneity in response to these vasoactive agents across different vascular beds.

Materials and Methods

Solutions and chemicals

Physiological salt solution (PSS) had the following composition (in mmol/L): NaCl 119, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 1.5, KH_2PO_4 1.18, $MgSO_4 \cdot 7H_2O$ 1.17, ethylenediaminetetraacetic acid (EDTA) 0.027, and glucose 5.5, with pH adjusted to 7.4. Ca^{2+}-free PSS was similar to PSS except that $CaCl_2$ was replaced by 0.01 mmol/L ethylene glycol-bis(2-aminoethyl ether)-*N*,*N*,*N*′*N*′-tetraacetic acid (EGTA). K-PSS was prepared by replacing all sodium with an equimolar amount of potassium resulting in a total K^+ concentration of 125 mmol/L.

All chemicals were obtained from Sigma-Aldrich (St Louis, MO) except for sarafotoxin 6c which was obtained from PolyPeptide Group (Strasbourg, France). All vasoactive compounds were dissolved in distilled H_2O. Stock solutions of the drugs were stored frozen in small aliquots at –20°C and dilutions were prepared just before experimentation.

Animals

Male C57BL/6NTac mice (25–30 g, $N = 22$, Taconic Europe, Lille Skensved, Denmark) and male Sprague–Dawley rats (250–300 g, $N = 7$, Taconic Europe) were housed in our local animal facility in a temperature- and humidity-controlled environment with 12-h light and 12-h dark cycle and ad libitum access to standard chow and water. All animal procedures were carried out in accordance with national laws and guidelines and the Guide for the Care and Use of Laboratory Animals was followed.

Tissue preparation

Animals were euthanized by cervical dislocation with subsequent exsanguination. Rats were sedated with CO_2 prior to euthanization. The mesenteric arcade was immediately excised and immersed in ice-cold PSS (see composition above). Following pinning out the mesenteric arcade in a silicon-covered petri dish, MRA (mice: 2nd order, rats: 3rd order) were isolated and immersed in ice-cold PSS.

Vascular force measurement

MRA segments (~2 mm long) were mounted on two stainless steel wires (mice: 25 μm diameter, rats: 40 μm diameter) in an organ bath of a small vessel wire

myograph (DMT, Aarhus, Denmark). The two wires were connected to a force transducer and a micrometer, respectively, allowing continuous measurement of isometric force development. In the organ bath of the wire myograph, MRA were allowed to equilibrate in 37°C aerated (5% CO_2/95% O_2) PSS for 1 h.

Internal circumference–tension relationship studies

In a series of experiments, the relationship between the internal circumference (IC) and passive and active tension of MRA was investigated as originally described by Mulvany and Halpern (1977). The relationship was investigated in order to estimate the optimal IC for maximal active wall tension (ΔAWT, baseline subtracted tension, Newton per meter, N m^{-1}) development. Briefly, passive wall tension (PWT) was recorded in artery segments exposed to Ca^{2+}-free PSS (see composition above) during a stepwise increase in artery IC by adjusting the micrometer. At each step, ΔAWT development was calculated as the difference in wall tension when the artery segments were maximally relaxed in Ca^{2+}-free PSS and maximally contracted with a depolarizing bicarbonate buffer solution (K-PSS, see composition above) to which 10 μmol/L noradrenaline was added.

For each artery segment the ratio IC_0/IC_{100} was estimated in order to establish the optimal IC for maximal ΔAWT development. The IC_0/IC_{100} ratio used in the normalization procedure of small arteries was previously described by Mulvany and Halpern (1977). IC_0 defines the IC at which the artery developed its maximal ΔAWT, that is, ΔAWT_0, whereas IC_{100} defines the IC the artery would have had when subjected to a passive transmural pressure (P) of 100 mmHg. This is in accordance with the law of Laplace where $P = T/D$ in which T is wall tension and D represents artery diameter. Artery segments with an active transmural pressure at $IC_0 > 13.3$ kPa were included in the analysis (Mulvany and Halpern 1977). Data from the passive tension-IC study were fitted to the following exponential growth function:

$$PWT = (PWT)_0 \cdot e^{[k \cdot IC]},$$

where κ is an IC constant that relates to the slope of the passive tension-IC relationship. Data from the active tension-IC study were fitted to a Gaussian distribution function:

$$\Delta AWT = \text{Amplitude} \cdot \exp\left(-0.5 \cdot \left(\frac{IC - \text{mean}}{SD}\right)^2\right),$$

where amplitude is the height of the center of the distribution, mean is the IC value at the center of the distribution, and SD is a measure of the width of the distribution (GraphPad Prism version 6.02 for Windows, GraphPad Software, La Jolla, CA).

In vitro pharmacology

In another series of experiments, isolated mouse MRA segments were stretched to their optimal IC in a stepwise manner on the basis of the performed IC-tension relationship study ($IC_0 = 0.9 \cdot IC_{100}$). Each protocol was initiated by repetitive challenges of the artery segments with K-PSS (4 repetitions of 5 min) in order to test vessel viability and reproducibility of force development and to deplete perivascular sympathetic nerve terminals (Fouda et al. 1991). Subsequently, the pharmacodynamic characteristics of the artery segments in response to cumulatively increasing (half-log increments) concentrations of selected vasoactive compounds were determined. K-PSS was applied between the cumulative concentration–response curves to reduce desensitization (Sheykhzade and Nyborg 1998).

Vasoconstrictive responses to the following compounds were tested: the neurotransmitter noradrenaline (NA, 1 nmol/L–100 μmol/L), the selective α_1-adrenoceptor agonist phenylephrine (PE, 1 nmol/L–10 μmol/L), the peptide hormone angiotensin II (Ang II, 1 pmol/L–300 nmol/L), the selective endothelin ETB receptor agonist sarafotoxin 6c (S6c, 10 pmol/L–100 nmol/L), and the monoamine neurotransmitter 5-hydroxytryptamine (5-HT, 100 pmol/L–10 μmol/L).

Vasodilatory responses were assessed on top of a stable precontraction induced by 3–10 μmol/L NA or PE, corresponding to about 80% of the maximal response of the individual artery segment to the adrenergic agonist (EC_{80}). The following compounds were evaluated: the endothelium-dependent vasodilator carbachol (10 nmol/L–10 μmol/L), the selective voltage-gated potassium channel (Kv7) opener retigabine (10 nmol/L–30 μmol/L), and the endothelium-independent vasodilator, NO donor sodium nitroprusside (SNP, 10 pmol/L–10 μmol/L).

Endothelial function in the MRA segments was assessed by the vasodilation mediated by 10 μmol/L carbachol. In our study, experiments were performed on artery segments eliciting ≥50% vasodilation in response to 10 μmol/L carbachol (Cortes et al. 1996; Russell and Watts 2000; Chin et al. 2007).

For each artery segment, cumulative concentration–response relationships for the vasoactive compounds were analyzed by nonlinear logistic regression analysis (GraphPad Prism version 6.02 for Windows, GraphPad Software) as previously described (Sheykhzade et al. 2012). Sensitivity to agonists was expressed by pEC_{50} values ($-\log EC_{50}(M)$).

Table 1. Estimated parameters from internal circumference (IC)-tension relationship study of mouse and rat mesenteric resistance-sized arteries.

	Mouse	Rat	*t*-test
n (*N*)	14 (8)	19 (7)	–
IC_0 (μm)	526 ± 30.8	697 ± 16.0	**
IC_{100} (μm)	601 ± 39.4	830 ± 22.0	**
PWT_0 (N m^{-1})	0.74 ± 0.07	0.76 ± 0.06	NS
ΔAWT_0 (N m^{-1})	1.56 ± 0.11	3.47 ± 0.33	**
PWT-IC slope, κ (N $m^{-1}\cdot\mu m^{-1}$)	0.008 ± 0.0005	0.007 ± 0.0003	*
IC_0/IC_{100}	0.88 ± 0.02	0.84 ± 0.01	NS

IC_0 represents the IC where maximal active wall tension, ΔAWT_0, was developed, PWT_0 is passive wall tension at IC_0, and IC_{100} represents the IC the artery would have had when subjected to a passive transmural pressure of 100 mmHg. Values are presented as mean ± SEM with *n* = artery segments and *N* = animals. The statistical analysis was performed with an unpaired *t*-test with $*P < 0.05$, $**P < 0.01$, and NS, not statistically significant.

Data and statistical analysis

Responses were expressed as PWT (Newton per meter, N m^{-1}), ΔAWT (active tension: baseline subtracted tension, N m^{-1}), or % of precontraction. Results are presented as mean ± SEM with *n* = number of artery segments and *N* = number of animals. Differences in means between mice and rats were analyzed by unpaired *t*-test. A *P*-value < 0.05 was considered statistically significant.

Compliance with design and statistical analysis requirements

Statistical analysis (*t*-test) was only performed on groups of $n > 5$ (Table 1). For the length-tension studies, only artery segments exhibiting an active transmural pressure at $IC_0 > 13.3$ kPa were included in the analysis (Mulvany and Halpern 1977). For the in vitro pharmacology studies, artery segments were included if they produced marked and reproducible force development in response to repetitive challenges with K-PSS (4 repetitions of 5 min) and elicited ≥50% vasodilation in response to 10 μmol/L carbachol. The studies were randomized by selection of animals and organ baths of the myograph. Concentration-dependent vasodilatory responses of artery segments were normalized as percentage of precontraction to adjust for minor differences in innervation and diameter between the artery segments.

Results

IC-tension relationships in mouse and rat isolated MRAs

In the first series of experiments, an IC-tension relationship study was performed in mouse and rat isolated MRA. The purpose was to determine the optimal IC for maximal active tension development and hence the optimal normalization ratio (i.e., IC_0/IC_{100}) in mouse MRA for future normalization procedures. Furthermore, we aimed to compare it to that of rat MRA. Figure 1 depicts

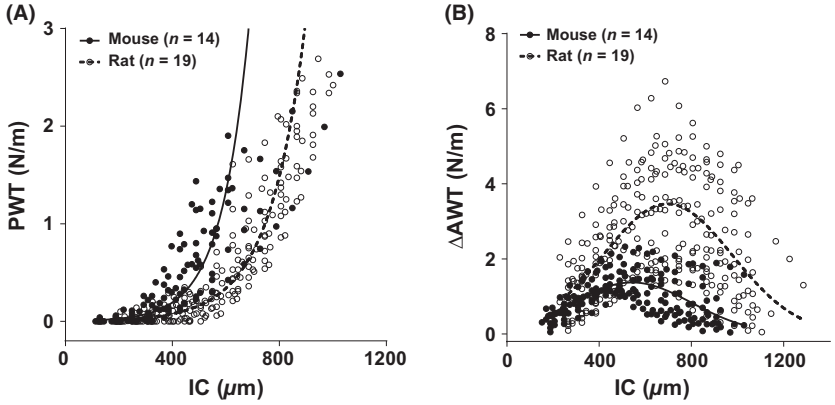

Figure 1. Internal circumference (IC)-tension relationships of mouse and rat mesenteric resistance-sized arteries (MRA). (A) Passive and (B) active tension-IC relationships of mouse 2nd order (filled labels and lines) and rat 3rd order (open labels and dashed lines) MRAs. The points represent each replicate artery segment fitted to (A) a mean exponential growth curve with $1/Y^2$ weighting and (B) a mean Gaussian distribution curve. See Table 1 for estimated parameters.

the relationships between IC and passive (Fig. 1A) and active (Fig. 1B) wall tension developed in mouse and rat MRA. The characteristics of the investigated MRA are shown in Table 1.

The passive tension of the artery segments at IC_0, that is, PWT_0, was similar in mouse and rat MRA. However, the constant κ of the passive tension-IC curve was significantly greater in mouse MRA compared to rat MRA, reflected by a steeper curve in mouse MRA (Fig. 1A).

The active tension-IC relationships of both mouse and rat MRA were shown to be bell-shaped around IC_0 (Fig. 1B). Maximal ΔAWT developed at IC_0 (ΔAWT_0) was significantly greater in rat MRA compared to mouse MRA. However, there was no significant difference in the estimated normalization ratios, IC_0/IC_{100}, when comparing MRA from mice and rats (Table 1).

Table 2. Characteristics of mouse mesenteric resistance-sized artery segments used for in vitro pharmacological characterization.

Parameter	
n (*N*)	22 (14)
IC_0 (μm)	602 ± 26.8
Tension in PSS (N m^{-1})	0.61 ± 0.05
Active tension in K-PSS (N m^{-1})	1.28 ± 0.09

Wall tension is expressed as Newton per meter, N m^{-1}. IC_0 equals $0.9 \cdot IC_{100}$ where IC_{100} represents the internal circumference the artery would have had when subjected to a passive transmural pressure of 100 mmHg. PSS refers to physiological salt solution, K-PSS is PSS where all sodium is replaced with with an equimolar amount of potassium resulting in a total K^+ concentration of 125 mmol/L. Tension in K-PSS represents the total wall tension minus wall tension in PSS. Values are expressed as mean ± SEM with *n* = artery segments and *N* = animals.

In vitro pharmacology

In the second series of experiments, a selected group of vasoactive agonists were investigated for their ability to constrict or dilate isolated MRA from C57BL/6 mice.

Basic mechanical properties of the investigated mouse MRA including normalized IC ($IC_0 = 0.9 \cdot IC_{100}$), tension in PSS, and K-PSS-mediated vasoconstriction are summarized in Table 2. Moreover, the estimated pEC_{50} values of the selected vasoactive agents in mouse MRA are summarized in Table 3. For comparison, Table 3 includes pEC_{50} values of the selected agonists from the literature, where available, estimated in mouse, rat, and human mesenteric arteries.

In terms of vasoconstrictive responses of mouse MRA, buffer containing a high potassium concentration (K-PSS, 125 mmol/L K^+) induced rapid and marked vasoconstriction. Cumulative concentration-dependent vasoconstrictive responses to selected vasoactive agents are depicted in Figure 2A. The adrenergic agonists NA and PE both induced marked concentration-dependent vasoconstriction of the isolated mouse MRA. In comparison to NA

Table 3. pEC_{50} values of selected vasoactive agents in mouse, rat, and human mesenteric arteries.

	Mouse				Rat		Human	
	Estimated		Literature		Literature		Literature	
Phenylephrine	6.35 ± 0.15	(6)	7.73	Longo et al. (2005)	5.65–6.2	Buus et al. (1994); Dhawan et al. (2004); Labruijere et al. (2013)		
Noradrenaline	5.68 ± 0.12	(9)	6.59	Kleinbongard et al. (2013)	5.77–6.79	Nielsen and Mulvany (1990); Buus et al. (1994); Hutri-Kahonen et al. (1999)	6.06–6.24	Muller-Schweinitzer et al. (1997); Hutri-Kahonen et al. (1999); Ferrero et al. (2013)
Sarafotoxin 6c	8.65 ± 0.14	(10)						
Carbachol	6.09 ± 0.11	(11)	5.59	Kleinbongard et al. (2013)	6.51–7.28	Vuylsteke et al. (2001); Wheal et al. (2012)	6.40*	Hutri-Kahonen et al. (1999)
SNP	7.81 ± 0.26	(5)	6.49–9.49	Hassanain et al. (2013); Kleinbongard et al. (2013)			6.72	Muller-Schweinitzer et al. (1997)
Retigabine	5.56 ± 0.09	(4)	5.52	Schleifenbaum et al. (2010)				

pEC_{50} represents the negative logarithm of the concentration of the agonist required to produce a half-maximal response. Values are presented as mean ± SEM with *n* = artery segments indicated between parentheses. Values estimated for sarafotoxin 6c are determined within the concentration range 10 pmol/L–30 nmol/L. "Estimated" refers to values obtained in our study in 2nd order mesenteric resistance-sized artery segments from C57BL/6 mice. "Literature" refers to pEC_{50} values in mouse, rat, and human mesenteric arteries described in the literature. SNP, sodium nitroprusside, *pEC_{50} of acetylcholine.

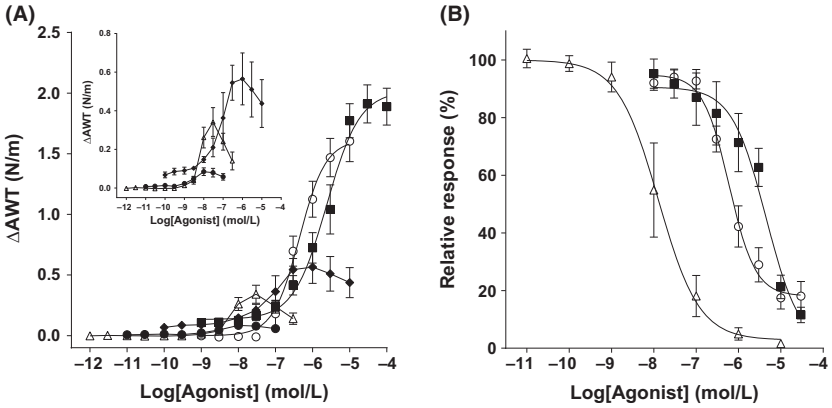

Figure 2. Cumulative concentration-dependent responses of mouse mesenteric resistance-sized arteries to selected vasoactive agents. Data are expressed as mean ± SEM with *n* = artery segments and *N* = animals in brackets. Cumulative concentration-dependent responses of mouse 2nd order mesenteric resistance-sized arteries (MRA) to (A) phenylephrine (open circle, *n* = 6 [4]), noradrenaline (filled square, *n* = 10 [6]), angiotensin II (open triangle, *n* = 16 [9]), sarafotoxin 6c (filled circle, *n* = 16 [9]), and 5-HT (filled diamond, *n* = 4 [2]) (Inset figure provides fine-scale resolution for responses to Ang II, S6c, and 5-HT) and (B) carbachol (open circle, *n* = 11 [8]), retigabine (filled square, *n* = 4 [3]), and sodium nitroprusside (open triangle, *n* = 5 [3]). Vasoconstrictive responses are presented as active wall tension (*Δ*AWT, N m^{-1}) and vasodilation is presented as percentage of precontraction. The concentration-dependent responses are fitted to nonlinear regression curves where possible. See Table 3 for estimated pEC_{50} values.

and PE, Ang II, S6c, and 5-HT induced only subtle vasoconstriction (Fig. 2A and Fig. 3: representative traces of PE and Ang II-induced responses as examples). The cumulative concentration–response curves of Ang II, S6c, and 5-HT were bell-shaped displaying an initial subtle vasoconstriction followed by vasodilation at higher concentrations. Of note, responsiveness to Ang II, S6c, and 5-HT varied significantly between the artery segments. In some arteries, the absence of concentration-dependent responses yielded nonconverging logistic nonlinear regression of Ang II- and 5-HT-induced concentration–response curves, and thus, no pEC_{50} values could be estimated. Nonlinear regression analysis of the S6c-induced concentration–response curves was only possible for 10 out of 16 artery segments.

Responses of mouse MRA precontracted with an adrenergic agonist to carbachol, SNP, and retigabine are shown in Figure 2B. All three agents induced concentration-dependent vasodilation of the investigated artery segments, although SNP was comparibly more potent and efficacious.

Discussion

Internal circumference-tension relationship studies were performed with the aim of establishing optimal settings for normalization of mouse MRA in the wire myograph which has not previously been investigated. Instead, a normalization ratio of 0.9 has traditionally been used based on studies in rat MRA (Mulvany and Halpern 1977; Mulvany and Nyborg 1980). The diameter of the investigated rat MRA in our study is in agreement with those described in previous studies. In terms of passive mechanical properties, the passive tension–IC curve in mouse MRA was significantly steeper than that of rat MRA. The estimated slope in mouse MRA is in agreement with a study by Longo et al. (2005). The slope of the curve is a measure of the elasticity of the artery wall, that is, the elastic modulus. The elastic modulus is determined by measuring the stress (force/cross-sectional wall area) produced in response to an applied strain (the fractional change in circumference) (Mulvany and Aalkjaer 1990). The greater the elastic modulus, the stiffer is the artery. The mechanical properties of passive vessels are predominantly determined by the contribution of elastin and collagen. The shape of the passive curve is believed to be dominated by the elastic modulus of elastin at low strains and that of collagen at higher strains (van den Akker et al. 2010). A steeper passive curve could thus be indicative of relatively more connective tissue (i.e., stiffness caused by different levels of elastin and collagen) in the mouse artery segments (Warshaw et al. 1979). This (i.e., collagen/elastin ratio), however, has to be determined morphologically. On the other hand, since the elastic modulus is inversely related to the diameter of the artery (Hayashi et al. 1980), one could argue that the smaller diameter of mouse MRA relative to rat MRA is a likely explanation for the steeper passive tension–IC curve in mouse MRA compared to that of rat MRA.

The bell-shaped active wall tension development around IC_0 observed in both mouse and rat MRA has previously been described in rat mesenteric (Mulvany and

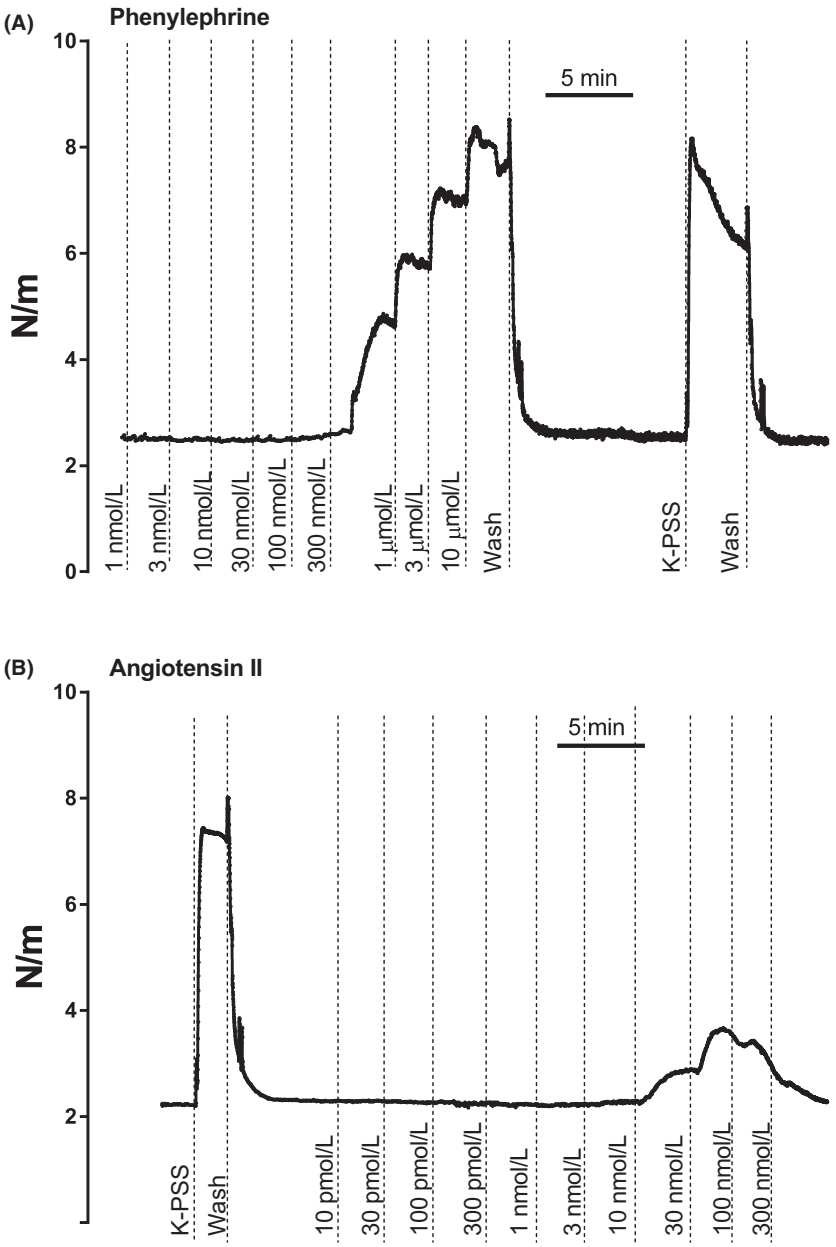

Figure 3. Representative traces of cumulative concentration-dependent responses of mouse mesenteric resistance-sized arteries to phenylephrine and angiotensin II. (A) phenylephrine, 1 nmol/L–10 μmol/L and (B) angiotensin II, 10 pmol/L–300 nmol/L.

Warshaw 1979) and coronary arteries (Sheykhzade et al. 2012). The greater maximal ΔAWT developed at IC_0 in rat MRA compared to mouse MRA is perhaps not surprising when taking the greater internal lumen diameter of rat MRA into account. In terms of the normalization ratio, the estimated IC_0/IC_{100} ratio in rat MRA in our study is in agreement with literature findings (Mulvany and Halpern 1977; Mulvany and Nyborg 1980). In conclusion, the IC-tension study showed that the optimal normalization ratio for mouse MRA was similar to that determined in rat MRA. To our knowledge, there are no corresponding studies in human mesenteric arteries. Although, a study in human omental arteries supports a normalization ratio of 0.9 (Aalkjaer and Mulvany 1981).

With the aim of elucidating the translational value of pharmacological studies in mouse isolated MRA, the estimated pEC_{50} values for a selected range of vasoactive agents were related to pEC_{50} values in the literature for the same vascular bed in mouse, rat, and human. In general, caution is needed when comparing pEC_{50} values

with those in the literature due to possible differences in the technique, buffer composition, level of precontraction and precontraction agent utilized as well as endothelial integrity, etc. (Buus et al. 1994; Falloon et al. 1995). The comparisons are therefore performed with this caution in mind. In addition, we have related the overall profile of the responses of mouse MRA to those of other arteries in a qualitative manner (i.e., subtle or marked vasoconstrictor/vasodilator response).

Our results on a rapid and marked potassium-mediated constriction (125 mmol/L K+) of mouse MRA are supported by studies in mouse (Kleinbongard et al. 2013), rat (Toma et al. 1995), and human mesenteric arteries (Stanley and O'Sullivan 2014). Furthermore, the concentration-dependent vasoconstrictions of mouse MRA mediated by NA and PE are in line with observations in C57BL/6 mouse thoracic (Russell and Watts 2000; Kleinbongard et al. 2013) and abdominal aorta, carotid, femoral, mesenteric, renal, and coronary arteries (Kleinbongard et al. 2013). The estimated potencies of NA and PE in our study are lower than potencies reported in similar studies on mouse mesenteric arteries (Longo et al. 2005; Kleinbongard et al. 2013). This might be explained by differential anatomical origin of the investigated artery segments as this can be a determinant of adrenoceptor distribution (i.e., receptor subtype and density) or by differential sympathetic innervation (Guimaraes and Moura 2001). Interestingly, our estimated potencies of adrenergic agonists in mouse MRA are in agreement with results obtained in rat mesenteric arteries (Nielsen and Mulvany 1990; Buus et al. 1994; Dhawan et al. 2004; Labruijere et al. 2013) and slightly lower than those reported from studies on human mesenteric arteries (Muller-Schweinitzer et al. 1997; Hutri-Kahonen et al. 1999; Ferrero et al. 2013).

In this study, it was not possible to estimate the potency of Ang II since the concentration–response curves could not be fitted to nonlinear regression. Thus, a quantitative comparison with literature data was not possible. Nevertheless, the subtle vasoconstrictive properties of Ang II in mouse MRA observed in our study are in line with findings in C57BL/6 mouse thoracic aorta (Russell and Watts 2000; Zhou et al. 2003) and carotid arteries (Zhou et al. 2003) as well as in rat mesenteric arteries (Juul et al. 1987; Falloon et al. 1995). Conversely, Ang II induced marked vasoconstriction in C57BL/6 mouse abdominal aorta and femoral arteries (Zhou et al. 2003) and in rat thoracic aorta and superior mesenteric arteries (Chen et al. 1995; Russell and Watts 2000). Moreover, the bell-shaped or biphasic profile of the Ang II-induced responses in our study is in accordance with previous observations in C57BL/6 mouse abdominal aorta, femoral and carotid arteries (Zhou et al. 2003), and rat small mesenteric arteries (Falloon et al. 1995; Andriantsitohaina et al. 1996). Indeed, regional differences in the vasoconstrictive responses to Ang II seem to be present (Juul et al. 1987). Moreover, the magnitude of Ang II-induced vasoconstriction in vitro has been shown to be dependent on methodology (i.e., pressurized versus wire myograph) (Falloon et al. 1995) as well as vascular tone (Juul et al. 1987). Possible explanations for the subtle bell-shaped vasoconstriction observed in the investigated mouse MRA may be degradation of Ang II, low density and/or presence of different subtypes of AT_1 receptors. Furthermore, rapid receptor desensitization/tachyphylaxis could play a role as suggested in mouse abdominal aorta, femoral arteries, and carotid arteries (Zhou et al. 2003). Finally, the presence of putative vasodilatory AT_2 receptors could mask the vasoconstrictive responses mediated through AT_1 receptors (Zhou et al. 2003) supported by a study showing the expression of both AT_1 and AT_2 receptors in C57BL/6 mouse MRA (Su et al. 2008).

The very subtle, if any, vasoconstriction mediated by the selective ETB receptor agonist S6c in isolated mouse MRA is in concordance with previous findings in mouse mesenteric arteries (Perez-Rivera et al. 2005), rabbit and rat mesenteric arteries (Iwasaki et al. 1999), rat coronary arteries (Skovsted et al. 2012), rat cerebral arteries (Hansen-Schwartz et al. 2002), and human arteries of variable sizes (Riezebos et al. 1994; Maguire and Davenport 1995). To our knowledge, there are no previous estimates of the potency of S6c in mouse mesenteric arteries. Moreover, the observed variability in responses to S6c between artery preparations has been described in rat mesenteric arteries (Mickley et al. 1997) and human arteries of various sizes (Maguire and Davenport 1995). Explanations for the variable responses to S6c could be differential receptor expression across the vascular bed or variations in intrinsic tone and endothelial function (Mickley et al. 1997). The S6c-induced concentration–response curves were, as observed for Ang II, bell-shaped. In agreement, in vivo studies in cat superior mesenteric arteries showed that S6c produced both vasodilation and vasoconstriction in this particular vascular bed (Minkes et al. 1992). The dual role exerted by ETB receptors located on vascular smooth muscle cells and endothelial cells on vascular tone could possibly explain this phenomenon (Schneider et al. 2007). Indeed, removal of the endothelium in rat mesenteric arteries increased the vasoconstrictive responses to endothelins and endothelin-like peptides including sarafotoxin 6b (Douglas and Hiley 1990).

In agreement with previous studies in mouse mesenteric arteries of larger IC (Kleinbongard et al. 2013) and aortic segments (Russell and Watts 2000), 5-HT mediated subtle bell-shaped vasoconstriction in mouse isolated MRA. In comparison, the vasoactive effects of 5-HT in

rat isolated artery segments remain controversial. Different studies report that 5-HT induced marked vasoconstriction in rat thoracic aorta and MRA (Watts 2002) and conversely induced subtle vasoconstriction in rat MRA (Davis et al. 2012). The vasoactive effects of 5-HT have, indeed, been described as very heterogeneous with regard to vascular type and size, species, and responsible receptor subtype (Watts et al. 2012). In mouse thoracic aorta, 5-HT-induced vasoconstriction was primarily mediated via the 5-HT2A receptor (McKune and Watts 2001) similar to rat thoracic aorta and MRA (Watts 2002). Yet, in rat MRA there is no evidence for a direct vasodilatory effect of 5-HT (Davis et al. 2012). To our knowledge, no corresponding studies have been performed in mouse MRA.

The estimated potency of the endothelium-dependent vasodilator carbachol in mouse MRA was rather similar to those obtained in previous studies using mouse and rat mesenteric arteries (Vuylsteke et al. 2001; Wheal et al. 2012; Kleinbongard et al. 2013). On the other hand, while a study of human isolated mesenteric arteries reported a sensitivity to acetylcholine similar to our finding, the responsiveness of human mesenteric arteries to the endothelium-dependent vasodilator was clearly less marked compared to rat mesenteric arteries of corresponding size (Hutri-Kahonen et al. 1999). Despite slight differences in sensitivities, the endothelium-independent vasodilation mediated by SNP in mouse MRA is supported by previous studies in mouse mesenteric arteries of various sizes (Hassanain et al. 2013; Kleinbongard et al. 2013), rat (Tesfamariam and Halpern 1988; Martinez-Revelles et al. 2008), and human mesenteric resistance arteries (Muller-Schweinitzer et al. 1997). Finally, the concentration-dependent vasodilation mediated by retigabine in mouse MRA is in accordance with previous findings in mouse (Yeung et al. 2007; Schleifenbaum et al. 2010), rat (Jepps et al. 2011), and human mesenteric arteries (Ng et al. 2011).

In conclusion, we found that the optimal normalization ratio, IC_0/IC_{100}, used for the important normalization procedure in wire myograph experiments was not significantly different in mouse and rat isolated MRA. Furthermore, the observed sensitivities to as well as the overall profile of the responses produced by NA, PE, S6c, carbachol, SNP, and retigabine in mouse isolated MRA were rather similar to those described in the literature for rat MRA and arteries of different sizes and species origin. The responses of mouse MRA to 5-HT and Ang II, however, were discrepant with findings in some, but not all, arteries of different sizes and species origin. This was mainly due to heterogeneity in response to these vasoactive agents across different vascular beds.

Overall, the results of this study provide a framework for evidence-based normalization of mouse MRA in the isometric wire myograph setup. Additionally, in terms of translational value, our study suggests that mouse MRA can be applied as a useful model for studying vascular reactivity. With the increasing use of mice in vascular research, we believe this is highly valuable knowledge for future studies of mouse MRA.

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Disclosures

None declared.

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