Vascular Reactivity of Isolated Thoracic Aorta of the C57BL/6J Mouse

AMBER RUSSELL and STEPHANIE WATTS
Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan
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ABSTRACT
We characterized the thoracic aorta from the C57BL/6J mouse, a strain used commonly in the generation of genetically altered mice, in response to vasoactive substances. Strips of aorta were mounted in tissue baths for measurement of isometric contractile force. Cumulative concentration-response curves to agonists were generated to observe contraction, or relaxation in tissues contracted with phenylephrine or prostaglandin F

\[ \text{PGF}_{2\alpha} \] in endothelium-denuded strips, the order of agonist contractile potency \(-\log EC_{50} [\text{M}]\) was norepinephrine > phenylephrine > 5-hydroxytryptamine > dopamine > \text{PGF}_{2\alpha} > \text{isoproterenol} > \text{KCl}. Angiotensin II and endothelin-1 were weakly efficacious (15% of maximum phenylephrine contraction), as were UK14,304, clonidine, histamine, and adenosine. In endothelium-intact strips, agonists still caused contraction and both angiotensin II and endothelin-1 remained ineffective. In experiments focusing on angiotensin II, angiotensin II-induced contraction was abolished by the AT\(_1\) receptor antagonist losartan (1 \(\mu\)M) but was not enhanced in the presence of the AT\(_2\) receptor antagonist PD123319 (0.1 \(\mu\)M), tyrosine phosphatase inhibitor orthovanadate (1 \(\mu\)M) or when angiotensin II was given noncumulatively. Prazosin abolished isoproterenol-induced contraction and did not unmask isoproterenol-induced relaxation. Angiotensin II and endothelin-1 did not cause endothelium-dependent or -independent relaxation in phenylephrine- or \text{PGF}_{2\alpha}-contracted tissues. Acetylcholine but not histamine, dopamine, or adenosine caused an endothelium-dependent vascular relaxation. These experiments provide information as to the vascular reactivity of the normal mouse thoracic aorta and demonstrate that the mouse aorta differs substantially from rat aorta in response to isoproterenol, angiotensin II, endothelin-1, histamine, and adenosine.

For years, the rat has served as a valuable model for studies in cardiovascular disease. With the advent of genomic manipulation, the mouse is at the forefront of use in scientific investigation. Herein, we establish normal vascular responses to a group of vasoactive substances in the thoracic aorta isolated from the C57BL/6J mouse, a mouse used commonly in the creation of genetically altered mice. Although significant effort has been made previously to examine the role of the endothelium and endothelial cell-derived vasoactive factors in mouse vasculature (Abe et al., 1998, Akishita, 1999; Faraci and Sigmund, 1999), a study of mouse vascular reactivity to contractile and relaxant agonists has not been previously performed. It should be noted that this series of studies was not meant to be nor is it exhaustive in terms of investigating all substances that can alter arterial smooth muscle tone. However, the group of agonists examined are representative of several important vasoactive systems. We found that the mouse aorta contracted to \(\alpha\)-adrenergic, serotoninergic, dopaminergic, and prostaglandin (PG) receptor agonists and, as has been observed in tissues from the rat, relaxed to acetylcholine in an endothelium-dependent manner. Surprisingly, the mouse aorta contracted with significantly weak efficacy to angiotensin II and endothelin-1, two peptide hormones with significant potency in the cardiovascular system of the rat. Because of our laboratory’s interest in hypertension, we performed a preliminary investigation in some of the mechanisms that might explain the lack of response to angiotensin II. Our focus on this particular agonist should not detract from the finding that other agonists, such as isoproterenol, histamine, and adenosine, did not act in the mouse aorta in a fashion similar to that observed in the rat aorta. Thus, there are significant differences in the vascular responsiveness of the rat and mouse aorta.

Materials and Methods

Isolated Tissue Bath Protocol. All animal procedures were followed in accordance with institutional guidelines established by Michigan State University. Male C57BL/6J mice (16–18 g; Jackson Laboratories, Bar Harbor, ME; carbon dioxide) or male Sprague-Dawley rats (250–300 g; Charles River Laboratories, Indianapolis, IN; pentobarbital [60 mg/kg i.p.]) were euthanized and thoracic aortae removed. Arteries were dissected into helical strips (mouse:

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ABBREVIATIONS: PG, prostaglandin; 5-HT, 5-hydroxytryptamine; AT, angiotensin; SNAP, S-nitroso-N-acetylpenicillamine.
0.15 × 0.75 cm; rat: 0.2 × 1.0 cm) and, in some experiments, the endothelial cell layer removed by rubbing the luminal side of the vessel with a moistened cotton swab. Tissues were placed in physiological salt solution for measurement of isometric contractile force with standard bath procedures. Physiological salt solution contained 130 mM NaCl, 4.7 mM KCl, 1.18 mM KH2PO4, 1.17 mM MgSO4·7H2O, 1.6 mM CaCl2·2H2O, 14.9 mM NaHCO3, 5.5 mM dextrose, and 0.03 mM CaNa2EDTA. One end of the preparation was attached to a stainless steel rod, the other was attached to a force transducer (FT03; Grass Instruments, Quincy, MA). Muscle baths were filled with warmed (37°C), aerated (95% O2, 5% CO2) physiological salt solution. Changes in isometric force were recorded on a Grass polygraph (Grass Instruments).

**Determination of Optimal Resting Tension.** Mouse aortic strips were placed under a particular tension by means of a rack and pinion, allowed to equilibrate for 30 min with buffer exchanges every 10 min, and then challenged with a maximal concentration of KCl (100 mM). Active force generation was recorded, tissues were washed for 30 min, and the passive tension placed on the tissues was increased. This procedure was repeated multiple times from passive tensions of 50 to 400 mg so as to generate a passive-active tension curve for determination of the optimal passive tension under which tissues should be placed. Such an experiment had been done previously for rat aortic strips and 1500 mg of tension was determined as optimal for this tissue.

**Determination of Agonist-Induced Mouse Thoracic Aortic Contraction.** Tissues equilibrated for 1 h under optimal tension. Tissues were challenged with a maximal concentration of phenylephrine (10−5 M). This contraction to phenylephrine within each experimental grouping was not different (~100 mg), and thus this response to phenylephrine was used to normalize contractile data. Tissues were washed and the status of the endothelium was examined by observing arterial relaxation to the endothelium-dependent agonist acetylcholine (1 × 10−6 M) in tissues contracted by a half-maximal concentration of the α1-adrenergic receptor agonist phenylephrine (1 × 10−7 M). Tissues were then washed multiple times and one of the following agonists was added in a cumulative fashion (from 10 μM to 30 μM) to generate a concentration-response curve: angiotensin II, endothelin-1, norepinephrine, phenylephrine, 5-hydroxytryptamine (5-HT), clonidine, UK14,304, dopamine, isoproterenol, PGF2α, KCl, histamine, or adenosine. After the cumulative response had reached a maximum, tissues were washed for 1 h (washes every few minutes) and a cumulative concentration-response curve was performed to a second agonist. The order in which agonists were tested was random except for endothelin-1. Because endothelin-1-induced contraction, even though small, was difficult to wash out, a second curve was not generated in these tissues. Tissues in which no contraction to the test agonist was observed were rechallenged with phenylephrine (10 μM) to ensure that the tissues were still viable.

In one series of experiments, the effects of the angiotensin AT2 receptor antagonist PD123319 was examined for its ability to unmask angiotensin II-stimulated contraction in the mouse aorta. In these experiments, either vehicle (water) or PD123319 (100 nM) was allowed to incubate with the aortic strips before cumulative addition of angiotensin II. In a separate series of experiments, a high concentration of angiotensin II (1 μM) was given to tissues never before exposed to angiotensin II to determine whether the process of performing a cumulative concentration-response curve minimized the maximal response to angiotensin II due to receptor and tissue desensitization. Some of the tissues in this series of experiments also were incubated with vehicle, the AT1 receptor antagonist losartan (1 μM), PD123319 (100 nM), or the tyrosine phosphatase inhibitor orthovanadate (1 μM). The last two manipulations inhibit direct activation and functioning of the AT2 receptor, respectively (Tsuzuki et al., 1996).

In similar experiments, the thoracic aorta (denuded of endothelium) from Sprague-Dawley rats was mounted in tissue baths to compare the contraction elicited by cumulative angiotensin II and endothelin-1 to that observed in the mouse aorta. These experiments were carried out in a manner similar to those described above.

**Determination of Agonist-Induced Mouse Thoracic Aortic Relaxation.** Agonists that did not elicit contraction in the mouse aorta or that we anticipated would cause relaxation were examined for their ability to stimulate arterial relaxation. Tissues equilibrated for 1 h under optimal tension and were challenged with a maximal concentration of phenylephrine (10−5 M). Tissues were washed and the status of the endothelium was examined as described above. Tissues were then washed multiple times and contracted again with an EC50 concentration of phenylephrine or PGF2α (5 μM). When contraction to these agonists was established, one of the following agonists was added in a cumulative fashion (from 10 μM to 30 μM) to generate a concentration-response curve: angiotensin II, endothelin-1, dopamine, histamine, adenosine, and acetylcholine. Isoproterenol was examined only in tissues contracted with PGF2α and, in some experiments, tissues were incubated with vehicle (methanol) or prazosin (100 nM) for 1 h before addition of PGF2α. Only one concentration-response curve was performed in tissues.

**Data Analysis.** Data are presented as mean ± S.E. and as a percentage of the initial response to maximal phenylephrine (10−5 M) or as a percentage of the contraction to phenylephrine (100 nM) or PGF2α (5 μM) for the number of animals indicated in parentheses. Agonist EC50 values were calculated with a nonlinear regression analysis with the algorithm [effect = maximum response/1 + (EC50/agonist concentration)] in the computer program GraphPad Prism (San Diego, CA). In instances where it appears that a maximum was not obtained in the concentration range tested, the EC50 value stated is an estimate and the true EC50 value is either equal to or greater than the value stated.

**Chemicals.** Solutions of compounds were prepared in deionized water unless indicated otherwise. Chemicals were obtained from the following sources: acetylcholine chloride, adenosine hydrochloride, angiotensin II, dopamine hydrochloride, histamine, 5-HT hydrochloride, isoproterenol, norepinephrine hydrochloride, phenylephrine hydrochloride, prazosin, [Sar1]angiotensin II, and sodium orthovanadate (Sigma Chemical Co., St. Louis, MO); endothelin-1 (Peninsula Laboratories, San Carlos, CA); PGF2α (ethanol; Biomol, Plymouth Meeting, PA); and clonidine, S-nitroso-N-acetylpenicillamine (SNAP; ethanol), PD123319, and UK14,304 (Sigma RBI, Natick, MA).

**Results**

A length-tension relationship was first performed to establish the passive tension at which aortic strips from the C57BL/6J mouse performed optimally under active stimulus. Figure 1 depicts the findings that a passive tension of more
than 200 mg results in an optimum and maximal contraction to KCl. A passive tension of 250 mg was used in all following experiments.

In the first series of experiments, we examined a group of vasoactive drugs for their ability to contract mouse aorta with and without intact endothelium. Figure 2 depicts results from aortic strips without the endothelial cell layer, strips in which acetylcholine caused less than a 5% relaxation of a phenylephrine (100 nM)-induced contraction. Agonists of the \( \alpha_1 \)-adrenergic receptor, phenylephrine and norepinephrine, were potent and efficacious agents, as was 5-HT. The \( \alpha_2 \)-adrenergic receptor in the mouse aorta appears to be the primary adrenergic receptor as the full \( \alpha_2 \)-adrenergic receptor agonist UK14,304 and partial \( \alpha_2 \)-adrenergic receptor agonist clonidine were both poorly efficacious. Isoproterenol also contracted the endothelium-denuded mouse aorta. Other agonists causing contraction of the mouse aorta were PGF\(_{2\alpha}\), dopamine, and depolarizing KCl. Agents producing a weak contraction included angiotensin II and endothelin; histamine and adenosine did not cause a measurable contraction.

Agonists that did not cause a contraction in the endothelium-denuded aortic strip as well as 5-HT (an agonist of interest to our laboratory), phenylephrine, and PGF\(_{2\alpha}\) (for later relaxant experiments) also were examined in tissues in which the endothelial cell layer was present (Fig. 3). This was determined because acetylcholine (1 \( \mu \)M) caused more than a 50% relaxation of strips contracted with an EC\(_{50}\) (\( \sim 30 \) nM; concentration necessary to cause a half-maximal effect) of phenylephrine. Table 1 compares the EC\(_{50}\) values of agonists in contracting endothelium-denuded versus endothelium-intact arterial strips. In general, tissues with intact endothelium were slightly but not significantly more sensitive to the agonists. Cumulative angiotensin II and endothelin-1 were poorly efficacious in tissues with and without an endothelial cell layer, as were adenosine and histamine. Angiotensin II and endothelin-1 produced a contraction between 15 and 20% of the maximum contraction produced by phenylephrine. This contrasts starkly with contraction stimulated by these agonists in isolated rat thoracic aorta (Fig. 4, top right).

**Table 1**  
Potency of agonists in contracting relaxing C57BL6/J mouse thoracic aorta.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Endothelium-Denuded</th>
<th>Endothelium-Intact</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contractile</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>9.16 ± 0.37 (9)</td>
<td>—</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>8.71 ± 0.17 (12)</td>
<td>9.16 ± 0.17 (6)</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>7.84 ± 0.03 (9)</td>
<td>ND</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>7.03 ± 0.11 (12)</td>
<td>7.45 ± 0.21 (4)</td>
</tr>
<tr>
<td>5-HT</td>
<td>7.00 ± 0.05 (8)</td>
<td>7.35 ± 0.21 (8)</td>
</tr>
<tr>
<td>Clonidine</td>
<td>7.04 ± 0.17 (6)</td>
<td>7.16 ± 0.15 (6)</td>
</tr>
<tr>
<td>UK14,304</td>
<td>6.14 ± 0.04 (8)</td>
<td>ND</td>
</tr>
<tr>
<td>Dopamine</td>
<td>5.96 ± 0.05 (6)</td>
<td>ND</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>5.89 ± 0.67 (4)</td>
<td>6.21 ± 0.05 (6)</td>
</tr>
<tr>
<td>PGF(_{2\alpha})</td>
<td>5.31 ± 0.50 (4)</td>
<td>5.14 ± 0.08 (4)</td>
</tr>
<tr>
<td>KCl</td>
<td>1.67 ± 0.26 (4)</td>
<td>ND</td>
</tr>
<tr>
<td>Histamine</td>
<td>— (10)</td>
<td>(4)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>Relaxant</td>
<td>(4)</td>
<td>6.90 ± 0.07 (9)</td>
</tr>
</tbody>
</table>

ND, not done.

* A value could not be obtained.

Because of our interest in hypertension, we next performed a small series of experiments that began to investigate the relatively poor contraction of the mouse thoracic aorta to angiotensin II. One possible explanation for a lack of contraction to angiotensin II in the mouse aorta may be the presence of the \( AT_2 \) receptor, a receptor described as opposing the functional effects of the \( AT_1 \) receptor. In the presence of the \( AT_2 \) receptor antagonist PD123319 (100 nM), angiotensin II still did not produce significant contraction in the mouse aorta (Fig. 4, top left). There was also no measurable contraction in the mouse aorta to an angiotensin II analog resistant to degradation by aminopeptidase(s), [\( \text{Sar}^1 \)]angiotensin II \( (10^{-11}-10^{-6} \) M; \( n = 4 \)). By contrast, rat aorta contracted to [\( \text{Sar}^1 \)]angiotensin II (1 \( \mu \)M) with a magnitude 71 ± 5% of a maximal phenylephrine contraction. In a final manipulation, one concentration of angiotensin II (1 \( \mu \)M) also was given to naïve tissues to determine whether desensitization played a role in the observed lack of significant mouse aortic contraction to angiotensin II. Figure 4

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*Fig. 2.* Agonist-induced contraction in the endothelium-denuded mouse thoracic aorta. Points represent means and vertical bars the S.E. for the number of animals indicated in parentheses. PE, phenylephrine.

*Fig. 3.* Agonist-induced contraction in the endothelium-intact mouse thoracic aorta. Points represent means and vertical bars the S.E. for the number of animals indicated in parentheses. PE, phenylephrine.

*Fig. 4.* Agonist-induced contraction in the endothelium-denuded mouse thoracic aorta. Points represent means and vertical bars the S.E. for the number of animals indicated in parentheses. PE, phenylephrine.
(bottom) depicts a representative tracing of the mouse aorta to a maximal concentration of angiotensin II, followed by a maximal concentration of phenylephrine (10 μM). As was observed in cumulative experiments, angiotensin II was weakly efficacious in the mouse aorta and produced a contraction 19 ± 2% of contraction to phenylephrine (10 μM). Contraction to angiotensin II (1 μM) in mouse thoracic aorta followed by a maximal concentration of phenylephrine (10 μM).

The next series of studies examined agonists for their ability to relax isolated mouse thoracic aorta. Figure 5 demonstrates that, as is found in many isolated arteries, acetylcholine caused a concentration- and endothelium-dependent relaxation of tissue contracted with phenylephrine; not shown is that the same occurs in tissue contracted with PGF2α.

None of the other agonists examined (endothelin-1, dopamine, histamine, angiotensin II, or adenosine) caused a significant relaxation of thoracic aorta (endothelium intact or denuded) contracted to a half-maximal level with phenylephrine (Fig. 6) and dopamine continued to elicit a contraction. The lack of aortic relaxation to both histamine and adenosine is a difference between the mouse and rat. Although not shown, similar results for all agonists were seen in tissues contracted with PGF2α.

As in tissues at baseline tone (Fig. 2), the β-adrenergic receptor agonist isoproterenol caused arterial contraction in tissues contracted with PGF2α (Fig. 7); isoproterenol-induced contraction was abolished by the α1-adrenergic receptor antagonist prazosin (100 nM), indicating that isoproterenol was stimulating α-adrenergic receptors to elicit contraction. These experiments were performed only in tissues contracted with PGF2α so as to avoid confounding interactions between β- and α-adrenergic receptors. Notably, isoproterenol-induced vasorelaxation was not unmasked in the presence of prazosin and these tissues are capable of relaxation because the nitric oxide donor SNAP (100 nM) completely relaxed PGF2α-contracted strips.
Mouse Thoracic Aorta: -Endothelium

Fig. 7. Effect of the $\alpha_1$-adrenergic receptor antagonist prazosin (100 nM) on isoproterenol-induced contraction in endothelium-denuded strips of mouse thoracic aorta. Points represent means and vertical bars the S.E. for the number of animals indicated in parentheses. * = statistically significant difference between the responses of strips with and without prazosin.

A final comparison for all data generated for angiotensin II in mouse aorta was done to determine whether angiotensin II-induced contraction was enhanced in the presence of either phenylephrine or PGF$_{2\alpha}$. Figure 8 shows results from both contractile and relaxant experiments for angiotensin II reported as a percentage of either phenylephrine or PGF$_{2\alpha}$-induced contraction. From a noncontracted baseline, angiotensin II induced no measurable contraction in tissues with endothelium, and induced an 11.9 ± 8.6-mg contraction in tissues when the endothelium was present. In the presence of the endothelium, angiotensin II-induced contraction was not observable in the presence of PGF$_{2\alpha}$, but was approximately 19 mg in the presence of phenylephrine. Statistically, neither phenylephrine nor PGF$_{2\alpha}$ potentiated the effects of angiotensin II.

**Discussion**

This study was undertaken to characterize the general vascular reactivity of the mouse aorta. An underlying hypothesis of this study was that the mouse aorta would respond similarly to that of the rat and, for many of the agonists examined, this is the case. However, vascular responsiveness to agonists of several receptor systems important to the cardiovascular system is significantly different.

Similar to the rat, the aorta from the mouse contracts to agonists of adrenergic, serotonergic, dopaminergic, and PG receptors. The primary adrenergic receptor subtype appears to be the $\alpha_1$-adrenergic receptor subtype because the $\alpha_2$-receptor agonists clonidine and UK14,304 were only minimally effective in stimulating contraction. Clonidine is a partial agonist in the rat aorta (Connolly et al., 1998; Iwanaga et al., 1998) and was a partial agonist in the mouse (Wong, 1997). UK14,304 is a full agonist at the $\alpha_2$-adrenergic receptor (Turner et al., 1985). The minimal role of $\alpha_2$-adrenergic receptors in the mouse aorta is in agreement with the findings of Mimura et al. (1995). These studies demonstrated that $\alpha_1$-, but not $\alpha_2$- or $\beta$-adrenergic agonists induced proliferation of smooth muscle cell cultures from the mouse aorta. Interestingly, isoproterenol contracted mouse aorta and did so through activation of the $\alpha_1$-adrenergic receptor because the $\alpha_1$-receptor antagonist prazosin completely blocked isoproterenol-induced contraction. Isoproterenol, however, did not stimulate relaxation and this is the first significant difference of note between rat and mouse arterial tissue (Chanman et al., 1999; Martin and Broadley, 1999; Trochu et al., 1999). We have not tested other agonists of the $\beta$-adrenergic receptor, but these data suggest that there may be no functional $\beta$-adrenergic system that is sensitive to isoproterenol in the mouse aorta. The mouse aorta is capable of relaxing to agonists because we observed relaxation to acetylcholine (endothelium-intact experiments) and tissues that did not relax to isoproterenol completely relaxed to the nitric oxide donor SNAP (100 nM).

The endothelial cell layer of the mouse aorta is functionally active because acetylcholine, a cholinergic agonist known well for its ability to activate nitric oxide synthase in endothelial cells of many other species, caused an endothelium- and concentration-dependent relaxation of tissues contracted with phenylephrine. We did not determine whether acetylcholine-induced relaxation was mediated by nitric oxide and/or prostacyclin, but this is an appropriate assumption to make because arteries from mice homozygous for the deletion of endothelial nitric oxide synthase do not relax to acetylcholine (Huang et al., 1995). The presence of the endothelium had little effect on the potency of contractile agonists.

Both histamine, which causes an endothelium-dependent relaxation in rat aorta (Lee et al., 1999), and adenosine, which relaxes rat aorta in an endothelium-independent manner (He et al., 1999), did not alter vascular tone. Newly purchased histamine and adenosine were used in all experiments, and the same solutions used in the mouse experiments demonstrated the appropriate response in rat aorta (data not shown). Thus, we cannot attribute a lack of mouse aortic response to histamine and adenosine to lack of drug efficacy. These findings suggest that, similar to the $\beta$-adrenergic receptor, histaminergic and adenosine receptor signaling in the mouse aorta is significantly different from that in the rat. One obvious possible explanation for this finding is that the appropriate receptors may not be present in the endothelial cell or smooth muscle cell, but this remains to be investigated.

Another surprising finding was lack of an efficacious response to two peptide hormones, angiotensin II and endothelin. Compared with the responses of aorta from the rat, mouse aorta responded poorly to both peptides. We focused on several possible mechanisms as to why angiotensin II-
induced contraction might be diminished. However, every tactic we took was not successful in improving mouse aortic contraction to angiotensin II. We know that the AT₂ receptor must be in mouse aorta smooth muscle because angiotensin II does cause a small contraction that is completely abolished by losartan. The noncumulative exposure to angiotensin II was done to determine whether the angiotensin II receptor in the mouse aorta desensitizes so rapidly that measurable contraction to angiotensin II is diminished (Sasamura et al., 1994). It can be argued that desensitization begins immediately on introducing angiotensin II to its receptor and that a true measure of contraction may therefore never be possible. If so, then desensitization occurs extremely rapidly (seconds). In general, the mouse has been described as a model of high circulating angiotensin II because angiotensin-converting enzyme inhibitors can reduce the blood pressure of wild-type mice (Oliverio et al., 1998). Thus, it is possible that arteries of the mouse are exposed to high circulating levels of angiotensin II and could thus be desensitized. This could not, however, be a complete desensitization nor would this necessarily be true for all the arteries of the mouse because when angiotensin I or angiotensin II is given to the mouse in vivo, blood pressure rises (Mattson and Krauski, 1998; Siragy et al., 1999a) and we do observe at least a small contraction. Our studies indicate that a large conduit vessel of the mouse does not respond to angiotensin II in a magnitude similar to that observed in the rat.

The mouse aorta may contain significant protease activity (Ikeda et al., 1999) that could rapidly destroy bioactive peptides. This idea was a reasonable because every peptide examined [angiotensin II, endothelin-1, and neuropeptide Y (data not shown)] had significantly poor efficacy. We approached this issue by testing the ability of the protease resistant analog of angiotensin II, [Sar₁]angiotensin II, to cause aortic contraction. This peptide caused no measurable contraction in the mouse aorta but did contract the rat aorta. Thus, it is unlikely that proteases are responsible for the lack of mouse aortic contraction to peptides. The AT₂ receptor antagonist PD123319 and tyrosine phosphatase inhibitor orthovanadate also did not improve either cumulative or noncumulative aortic response to angiotensin II. The purpose behind these experiments was to determine whether a concomitant activation of the AT² receptor masked contraction mediated by an AT₁ receptor. The AT₂ receptor has been described as a receptor that opposes the progrowth and contractile characteristics of the AT₁ receptor (Nakajima et al., 1995; Griendling et al., 1996, 1997). For example, activation of the AT₂ receptor appears to stimulate tyrosine phosphatase activity (Bottari et al., 1992), an activity that counteracts the well established activation of the tyrosine kinase-dependent extracellular signal-regulated kinase mitogen-activated protein kinase pathway (Berk and Corson, 1997; Griendling et al., 1997). Interestingly, mice lacking the AT₂ receptor (Agtr2–) demonstrate not only a pressor response to angiotensin II but also isolated rings from these animals respond to angiotensin II with a greater magnitude of contraction compared with wild type (Akishita et al., 1999; Tanaka et al., 1999). These mice have a different genetic background (FVB/N and 129/SV) but were similar in age (7–8 weeks) to the mice in this study. Thus, there is a possibility that different strains of mice respond differently to angiotensin II. However, another strain of AT₂ receptor null mice derived from older (12–16 weeks old) C57BL/6 mice displayed a slightly elevated blood pressure compared with wild type, suggesting that activation of the AT₂ receptor keeps blood pressure down (Siragy et al., 1999a,b). Tsutsumi et al. (1999) demonstrated the ability of angiotensin II to contract aortic strips from a C57BL/6 strain of transgenic mice but did not describe the efficacy of angiotensin II-induced contraction relative to other substances. Age also might influence vascular responsivity to angiotensin II because Viswanathan et al. (1991) demonstrated that, in the rat, the primary vascular smooth muscle angiotensin receptor was an AT₂ receptor in the young animals and switched to predominantly an AT₁ receptor population in rats 8 weeks of age. However, angiotensin II did not cause a significant concentration-dependent contraction in aorta isolated from C57BL/6 mice that are 16 weeks old (data not shown) nor the 7- to 8-week-old mice used in this study. Thus, these data suggest age may not be a factor. Finally, the lack of effect of both a direct receptor antagonist (PD123319) and an inhibitor of a signal transduction pathway by the AT₂ receptor (orthovanadate) can be interpreted to mean that the AT₂ receptor does not oppose activation of the AT₁ receptor in the mouse aorta.

In summary, this study provides what should be useful data for the pharmacologist and physiologist investigating the vasculature of the mouse. The mouse aorta was similar to that of the rat with respect to sensitivity to α₁-adrenergic, serotonergic, dopaminergic, and PG receptors but differed substantially from the rat in a lack of relaxation to the β-adrenergic agonist isoproterenol, adenosine, and histamine and in a lack of significant contraction to either angiotensin II or endothelin-1. It is now important to determine why these differences occur and the physiological impact they may have on the cardiovascular system.

References


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Send reprint requests to: Stephanie W. Watts, Ph.D., B445 Life Sciences Bldg., Department of Pharmacology & Toxicology, Michigan State University, East Lansing, MI 48824-1317. E-mail: wattss@msu.edu