

Effects of Angiotensin Metabolites in the Coronary Vascular Bed of the Spontaneously Hypertensive Rat

Loss of Angiotensin II Type 2 Receptor–Mediated Vasodilation

Els Moltzer, Anna V.A. Verkuil, Richard van Veghel, A.H. Jan Danser, Joep H.M. van Esch

Abstract—Because angiotensin (Ang) metabolites mediate functions independent of Ang II, we investigated their effects on coronary flow in spontaneously hypertensive rats (SHRs). Results were compared with those in the iliac artery and abdominal aorta and the coronary circulation of the Wistar rat. Ang II, III, and IV decreased coronary flow in SHRs and Wistar rats, with Ang III and IV being ≈ 10 and ≈ 1000 times less potent than Ang II. Ang-(1-7) decreased coronary flow at concentrations $>1 \mu\text{mol/L}$ in SHRs. The Ang II type 1 receptor antagonist irbesartan blocked the effects of Ang II, III, and IV, whereas the Ang II type 2 receptor antagonist PD123319 blocked the effects of Ang-(1-7). The maximal Ang II- and III-induced decreases in coronary flow in SHRs were twice as large as those in Wistar rats. PD123319 enhanced the constrictor effects of Ang II and III in Wistar rats so that, in the presence of this drug, their effects were comparable to those in SHRs. In contrast, PD123319 did not alter the Ang II- and III-induced responses in SHRs and blocked the constrictor effect of Ang II in iliac arteries. Ang II type 2 receptor–mediated relaxation did not occur in iliac arteries and abdominal aortas, and the constrictor effects of Ang metabolites in these vessels were identical in Wistar rats and SHRs. In conclusion, coronary constriction induced by Ang II, Ang III, and Ang-(1-7) is enhanced in SHRs as compared with Wistar rats. This is attributable to the absence of counterregulatory Ang II type 2 receptor–mediated relaxation and/or a change of the Ang II type 2 receptor phenotype from relaxant to constrictor. (*Hypertension*. 2010;55[part 2]:516-522.)

Key Words: angiotensin III ■ angiotensin (1-7) ■ AT₂ receptor ■ spontaneously hypertensive rat ■ Wistar rat

Angiotensin (Ang) I and II are metabolized by a whole range of peptidases,¹ resulting in the generation of Ang III, Ang IV, and Ang-(1-7). Ang II exerts its effects via Ang II type 1 (AT₁) and type 2 (AT₂) receptors, whereas Ang III, Ang IV, and Ang-(1-7) mediate functions of their own by stimulating AT₁, AT₂, and/or newly discovered receptors.²⁻⁷ For instance, Ang III appears to be the preferred agonist of the AT₂ receptor both in the heart and kidney, inducing, respectively, coronary vasodilation⁸ and natriuresis.⁵ In addition, Ang III regulates blood pressure via central AT₁ receptor activation.⁹ Ang IV mediates relaxant effects via Ang II type 4 receptors,¹⁰ whereas Ang-(1-7) activates vasodilatory Mas receptors.¹¹

AT₂ receptor upregulation and/or AT₁ receptor downregulation (resulting in a relative AT₂ receptor upregulation) is generally believed to induce protective effects under pathophysiological conditions.¹²⁻¹⁴ However, such beneficial effects have not been found consistently by all of the investigators. For instance, AT₂ receptors mediate constriction in the renal medulla of 2-kidney, 1-clip rats¹⁵ and in mesenteric arteries of spontaneously hypertensive rats (SHRs),¹⁶ and the AT₂ receptor-induced natriuresis by Ang III no longer occurs in SHRs.¹⁷ Interestingly, blood pressure-lowering in SHRs

restored the vasodilatory function of the AT₂ receptor.¹⁸ Chronic treatment of apolipoprotein E knockout mice with Ang IV reversed vascular dysfunction, possibly by enhancing NO bioavailability in an AT₂ and/or Ang II type 4 receptor–dependent manner.⁷ Finally, Ang-(1-7) exerts vasodepressor^{19,20} and anti-remodeling²¹ effects under pathological conditions. Although this has been attributed to its capacity to activate Mas receptors,²² it may also involve AT₂ receptor activation,²⁰ ACE inhibition,²³ and/or AT₁ receptor blockade.^{8,24}

Given the conflicting data regarding the endogenous agonist and effect(s) of the AT₂ receptor under pathophysiological conditions, here we compared the effects of Ang II, Ang III, Ang IV, and Ang-(1-7) in the coronary vascular bed, iliac artery, and aorta of the SHR under carefully standardized conditions, both with and without blockade of AT₁ or AT₂ receptors. These vascular beds were chosen because they had been studied previously in Wistar rats,⁸ thus allowing a detailed comparison of AT₂ receptor function between normotensive and hypertensive rats.

Methods

Animals

Sixty-three 3-month-old male SHRs (mean arterial blood pressure: 146 ± 3 mm Hg, $n=10$; body weight: 296 ± 3 g, $n=63$) were obtained

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from Charles River (Germany). All of the experiments were performed under the regulation and permission of the animal care committee of the Erasmus MC.

Tissue Collection

Male SHR were anesthetized with pentobarbital (60 mg/kg IP). Hearts were rapidly excised and placed in ice-cold Tyrode buffer,⁸ gassed with 95% O₂ and 5% CO₂. Subsequently, the iliac arteries and abdominal aorta were removed and either used directly or after overnight storage in cold, oxygenated Krebs-Henseleit solution.⁸ Overnight storage did not affect responsiveness.^{25,26}

Langendorff Preparation

Hearts were perfused according to Langendorff, as described before.⁸ Coronary flow (CF) was measured with a flow probe (Transonic Systems). After a stabilization period of 30 minutes, baseline values of CF were obtained. Next, bolus injections (100 μL) of Tyrode buffer were applied 3 times to determine injection-induced changes in CF. Subsequently, concentration-response curves to Angs were constructed by applying bolus injections in the absence or presence of 1 μmol/L of irbesartan or PD123319 in the perfusion buffer.^{6,27}

Mulvany Myographs

Iliac arteries (diameter: 954±8 μm; n=149) and abdominal aortas (diameter: 1279±15 μm; n=145) were cut into ring segments of ≈2-mm length and mounted in a Mulvany myograph with separated 6-mL organ baths containing gassed (95% O₂/5% CO₂) Krebs-Henseleit buffer at 37°C. No antioxidants were added. The tension was normalized to 90% of the estimated diameter at 100-mm Hg effective transmural pressure.²⁸ After a 30-minute stabilization period, the maximal contractile response was determined by exposing the vessels to 100 mmol/L of KCl. Thereafter, the vessels were preincubated for 30 minutes in fresh buffer in the absence or presence of 100 μmol/L of N^G-nitro-L-arginine methyl ester (L-NAME), 1 μmol/L of irbesartan, or 1 μmol/L of PD123319, and concentration-response curves to Angs were constructed. To study vasorelaxation, vessels were precontracted with U46619 (10 to 100 nmol/L) before their exposure to Angs.

Data Analysis

Data obtained with the Langendorff preparation were recorded and digitalized using WinDaq waveform recording software (Data Instruments). After a manual selection of the desired signals preinjection and postinjection, data were analyzed using Matlab (Mathworks Inc). Six consecutive beats were selected for CF determination. Concentration-response curves were analyzed as described before,²⁹ using GraphPad Prism 3.01 (GraphPad Software Inc), to determine the maximum effect and pEC₅₀ (= -¹⁰logEC₅₀) values. The pEC₅₀ values refer to the agonist concentration in injection fluid of the Langendorff model and do not reflect the actual concentrations seen by the receptor. In the Mulvany myograph studies, Ang III and Ang IV did not reach maximum effect at the highest concentrations used. We, therefore, determined the concentration required to obtain 5% of the K⁺-induced contraction (EC_{5%K⁺}) to calculate pEC_{5%K⁺} values.²⁵ Statistical analysis was by 2-way ANOVA, followed by post hoc evaluation according to Dunnett. *P*<0.05 was considered significant.

Results

Langendorff Preparation

Baseline CF (9.6±0.4 mL/min; n=60) was similar in all of the groups. Bolus injections with Tyrode buffer injections did not significantly affect CF (Figure 1). Ang II, Ang III, Ang IV, and Ang-(1-7) concentration-dependently decreased CF, by maximally 66±5%, 74±3%, 42±9%, and 24±3%, respectively (Figure 1A through 1D). Ang III (pEC₅₀ 6.9±0.1; n=6), Ang IV (pEC₅₀ 5.4±0.2; n=5), and Ang-(1-7) (pEC₅₀

5.0±0.3; n=4) were, respectively, ≈16-, ≈501-, and ≈1259-fold less potent (*P*<0.05 for all) than Ang II (pEC₅₀ 8.1±0.4; n=7). Irbesartan abolished all Ang II-, Ang III-, Ang IV-, and Ang-(1-7)-induced flow changes (n=4), whereas PD123319 abolished the constrictor effects of Ang-(1-7) only (n=4; *P*<0.05).

Mulvany Myographs

Ang II, Ang III, and Ang IV constricted iliac arteries (Figure 2A through 2H) and abdominal aortas (Figure 3A through 3H) in a concentration-dependent manner, whereas Ang-(1-7) had no effect. In the abdominal aorta, but not in the iliac artery, L-NAME greatly enhanced (*P*<0.05) the response to Ang II. In iliac arteries, in the presence of L-NAME, Ang III and Ang IV (pEC_{5%K⁺}: 7.3±0.2, n=5, and 5.5±0.2, n=4) were, respectively, ≈25- and ≈1584-fold (*P*<0.001 for both) less potent than Ang II (pEC_{5%K⁺}: 8.7±0.3; n=5). Potencies in the absence of L-NAME (n=5 to 6) were identical to those in the presence of L-NAME. In abdominal aortas (n=5 to 6), both with and without L-NAME, the potencies of Ang II, III, and IV were identical to those in iliac arteries.

Irbesartan abolished all of the Ang-induced contractions in the presence of L-NAME in both iliac arteries (n=5 to 7; Figure 2A through 2C) and abdominal aortas (n=4 to 10; Figure 3A through 3C) but did not unmask Ang-(1-7) effects (Figure 2D and 3D, respectively). Without L-NAME, PD123319 lowered the contractile response to Ang II (*P*<0.05; n=8 to 9; Figure 2E) in iliac arteries. It did not affect the responses to Ang III, Ang IV, and Ang-(1-7) in iliac arteries (n=4 to 8; Figure 2F through 2H), nor did it alter any of the responses in abdominal aortas (n=5 to 10; Figure 3E through 3H). No relaxant responses to Ang II, Ang III, Ang IV, or Ang-(1-7) were observed in precontracted iliac arteries or abdominal aortas in the absence or presence of irbesartan (n=4 to 6; data not shown).

Discussion

All of the Ang metabolites evaluated in this study caused coronary vasoconstriction in SHRs via AT₁ receptor stimulation. Although their potencies were identical to those in Wistar rats, the coronary constrictor efficacy of both Ang II and III was much larger in SHRs (Figure 1). PD123319 did not enhance the coronary effect of Ang II and III in SHRs, as opposed to its potentiating effects in the hearts of Wistar rats. In fact, the coronary constrictor effects of Ang II and III in SHRs in the absence of PD123319 were as large as their coronary constrictor effects in Wistar rats in the presence of PD123319 (Figure 1). This suggests that the main reason for the enhanced coronary constrictor effects in SHRs is the lack of counterregulatory AT₂ receptor-mediated coronary vasodilation. Such vasodilation is endothelium dependent and involves bradykinin B₂ receptor activation, endothelial NO synthase, NO, and cGMP.^{30–32} Both in the coronary circulation⁸ and the kidney,⁵ Ang III appeared to be the preferred agonist of the AT₂ receptor. The absence of this vasodilator effect in SHRs may relate to the endothelial dysfunction in this model, as observed in the coronary³³ and other vascular beds.^{34,35} This dysfunction is believed to be the result of enhanced reactive oxygen species production under pathologic

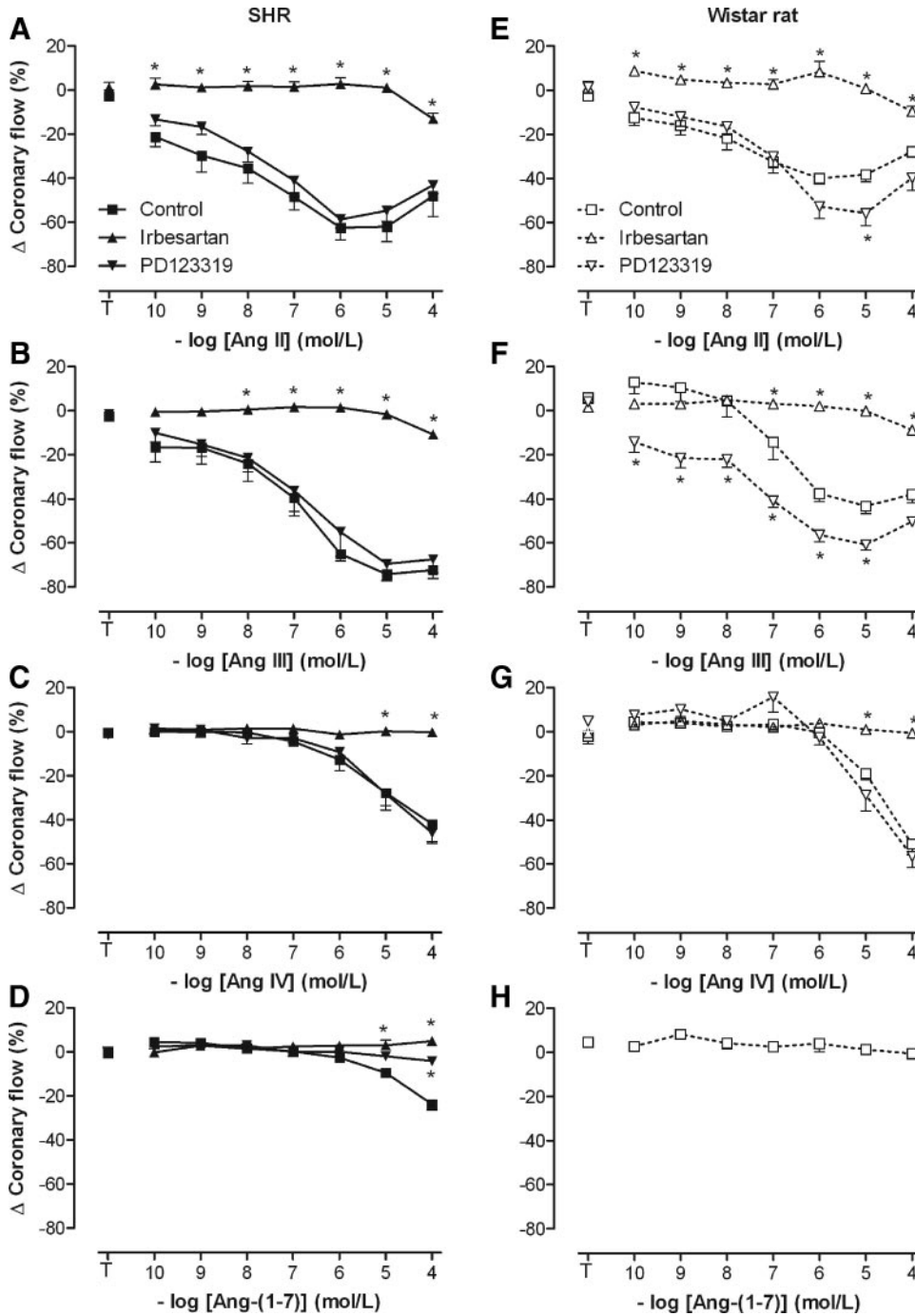


Figure 1. Effects of angiotensins on CF in SHR (left) and Wistar rats (right; redrawn from van Esch et al⁸) with or without irbesartan or PD123319. The x axis displays the concentration of the agonist in the injection fluid. Data (mean ± SEM of n=4 to 7) represent percentage change from baseline. T indicates bolus injection of Tyrode buffer. *P<0.05 vs control within the same strain.

ical conditions.³⁶ Reactive oxygen species may in fact directly downregulate AT₂ receptors.³⁷ If significant, it will no longer allow the previously described AT₁-AT₂ receptor heterodimerization, which is responsible, at least in part, for AT₂ receptor-mediated effects.^{27,38}

Alternatively, the phenotype and/or location of the AT₂ receptor may change under pathophysiological conditions. For instance, AT₂ receptor stimulation induced constriction in mesenteric arteries of SHR^{16,18} as opposed to relaxation in Wistar-Kyoto rats.¹⁸ Because this contractile response was

not affected by removal of the endothelium,¹⁸ the site of AT₂ receptor expression apparently had changed from the endothelium to the smooth muscle cell.

In the present study, Ang-(1-7) caused coronary constriction in SHR in an AT₂ receptor-dependent manner (Figure 1D), confirming that the function of this receptor in the coronary vascular bed had also changed from vasodilator to vasoconstrictor. Moreover, the Ang II-induced constriction of iliac arteries obtained from SHR partly involved AT₂ receptors (Figure 2E). Preliminary experiments in endothelium-denuded

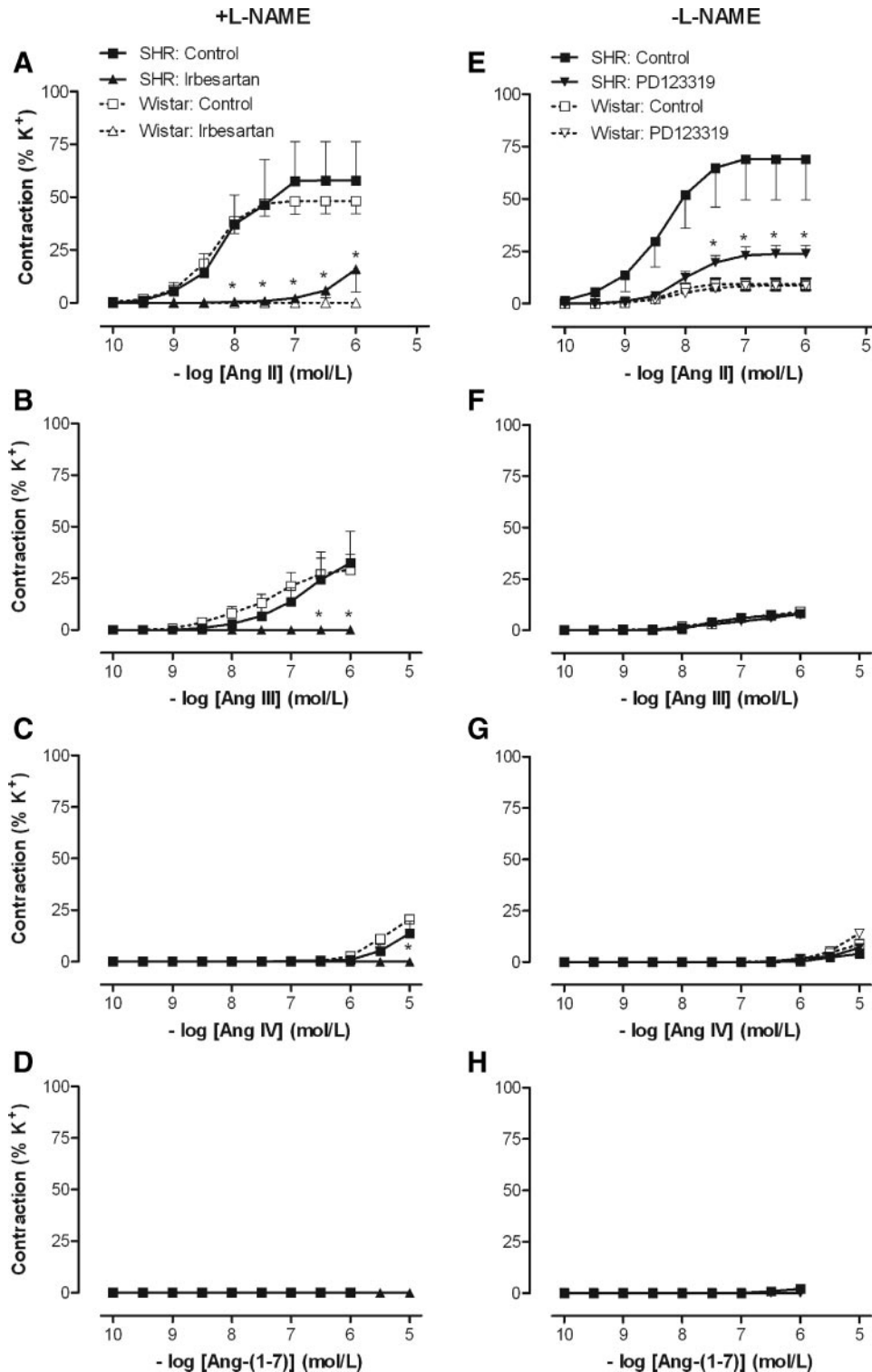


Figure 2. Effects of Angs in iliac arteries of SHRs and Wistar rats (redrawn from van Esch et al⁸) with or without L-NAME, irbesartan, or PD123319. The x axis displays the concentration of the agonist in the organ bath fluid. Data (mean±SEM of n=4 to 9) are expressed as a percentage of the response to 100 mmol/L of KCl. **P*<0.05 vs SHR control. The effects of irbesartan in Wistar rats were identical to those in SHRs.

iliac arteries (n=2; J.H.M. van Esch et al, unpublished data, 2009) furthermore revealed that this AT₂ receptor-mediated contractile response, like that in the SHR mesenteric artery,¹⁸ occurred in an endothelium-independent manner. Finally, in iliac arteries, L-NAME greatly enhanced the constrictor effect of Ang

II in Wistar rats (Figure 2A and 2E), whereas in SHRs the effect of Ang II in the absence of L-NAME was already as large as that in Wistar rats in the presence of L-NAME, with the addition of L-NAME causing no further effect. This illustrates the presence of endothelial dysfunction in SHRs, no longer

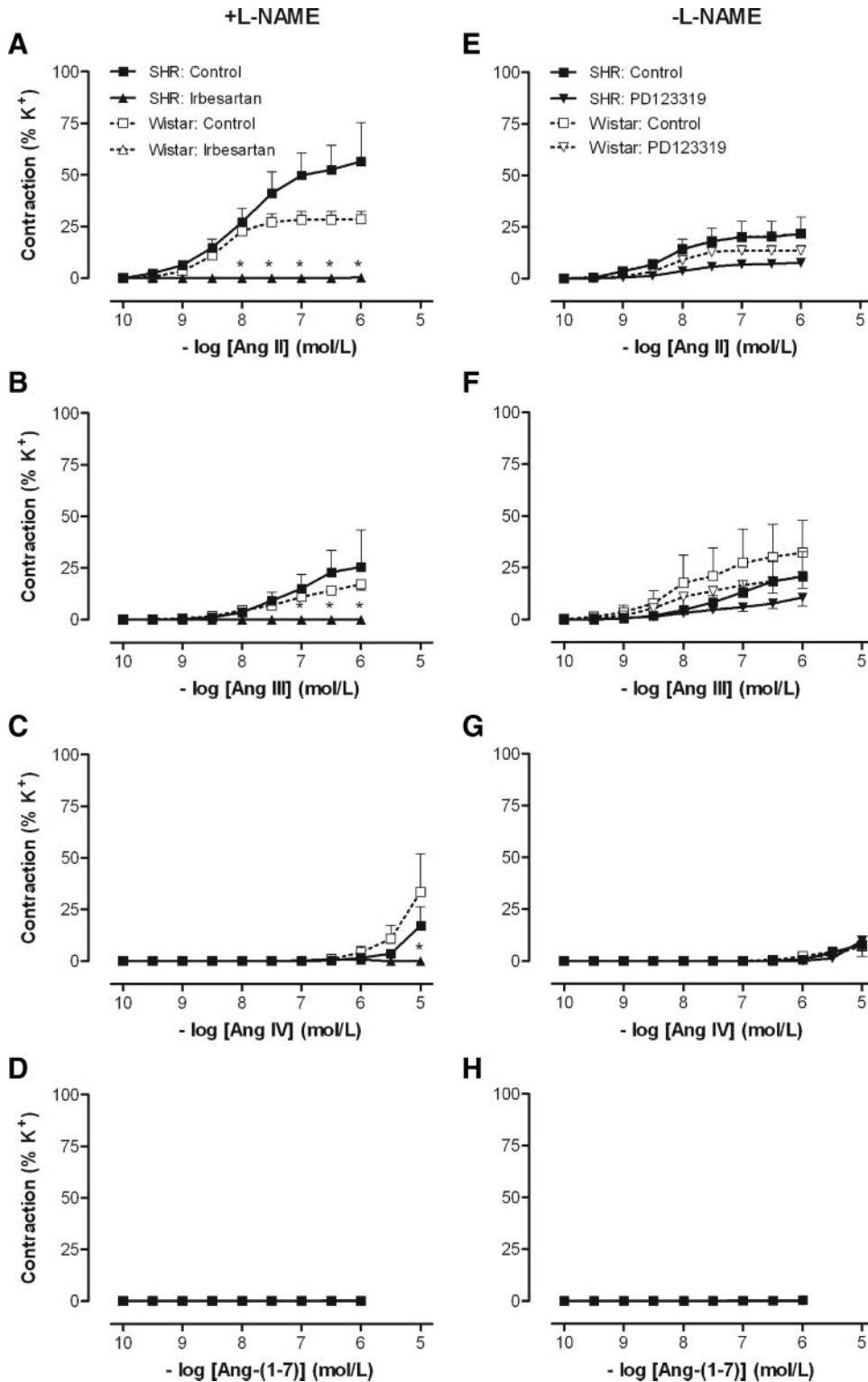


Figure 3. Effects of angiotensins in abdominal aortas of SHRs and Wistar rats (redrawn from van Esch et al⁸) with or without L-NAME, irbesartan, or PD123319. The x axis displays the concentration of the agonist in the organ bath fluid. Data (mean ± SEM of n=4 to 10) are expressed as a percentage of the response to 100 mmol/L of KCl. *P<0.05 vs SHR control. The effects of irbesartan in Wistar rats were identical to those in SHRs.

allowing endothelial NO to counteract Ang II-induced constriction. Such dysfunction had not yet occurred in the abdominal aortas of the SHRs because in these vessels L-NAME still enhanced the effect of Ang II, whereas

PD123319 exerted no (significant) blocking effect toward Ang II-induced vasoconstriction.

The constrictor effects of Ang III and IV in iliac arteries and abdominal aortas were much more modest than those of

Ang II, at least at concentrations $\leq 10 \mu\text{mol/L}$. No differences occurred between SHRs and Wistar rats, nor were these responses affected by L-NAME or PD123319 (Figures 2 and 3). The complete blockade of these effects by irbesartan suggests that they are exclusively AT₁ receptor mediated. Clearly, therefore, these modest responses reflect the reduced potency of Ang III and IV toward AT₁ receptors as compared with Ang II.^{39,40} Moreover, given the absence of Ang III-induced (constrictor) responses mediated via AT₂ receptors, it appears that Ang II is the preferred agonist of this AT₂ receptor-mediated constriction, as opposed to the relaxant effect of AT₂ receptor stimulation in normotensive animals, where Ang III is the preferred agonist.⁸

Under no condition did Ang-(1-7) exert constrictor or (after precontraction) dilator effects in the iliac arteries or abdominal aortas of either SHRs or Wistar rats. The vasodilator effects of Ang-(1-7) that have been described in vitro^{11,41} may, therefore, be limited to certain vascular beds.

In conclusion, Ang-induced coronary constriction is enhanced in SHRs as compared with Wistar rats, because the counterregulatory AT₂ receptor-mediated relaxant effects are either absent or have been reversed into constrictor effects. Similarly, in iliac arteries of SHRs, AT₂ receptors mediate contractile responses. In addition, the lack of endogenous endothelial NO production in these vessels greatly increases the response to Ang II.

Perspectives

AT₂ receptor function changes under hypertensive conditions from relaxant to constrictor, and Ang II rather than Ang III then becomes its endogenous agonist. It remains to be determined whether this is because of reactive oxygen species-induced endothelial dysfunction, resulting in the disappearance of endothelial (dilator) AT₂ receptors and/or alternative expression of (constrictor) AT₂ receptors in smooth muscle cells. It might also be the consequence of hypertension, per se, because blood pressure lowering reversed the AT₂ receptor-mediated vasoconstriction into vasodilation.¹⁸ The fact that AT₂ receptors are capable of inducing vasoconstriction raises concern regarding the clinical application of AT₂ receptor agonists like compound 21,⁴² at least in the absence of AT₁ receptor blockade. AT₂ receptor agonists would allow for a direct evaluation of AT₂ receptor function, thereby potentially overcoming the disadvantage of the indirect approach in the present study, which relies on the use of antagonists with limited specificity.

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Disclosures

None.

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