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Prostanoids, but not nitric oxide, counterregulate angiotensin II mediated vasoconstriction in vivo

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Abstract

To evaluate the modulating effects of nitric oxide and prostanoids during angiotensin II-mediated vasoconstriction, male Wistar rats ($n = 25$) were infused with increasing doses of angiotensin II following pretreatment with the cyclooxygenase inhibitor indomethacin, the nitric oxide-synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) plus sodium nitroprusside to restore mean arterial blood pressure, or saline. Hemodynamics were studied with the radioactive microsphere method. Indomethacin did not alter systemic or regional hemodynamics. L-NAME + sodium nitroprusside reduced cardiac output, as well as systemic and renal vascular conductance. Angiotensin II increased mean arterial blood pressure and heart rate, and decreased systemic vascular conductance as well as vascular conductance in gastrointestinal tract, kidney, skeletal muscle, skin, mesentery + pancreas, spleen and adrenal. Indomethacin enhanced the angiotensin II-mediated effects in all vascular beds, whereas L-NAME + sodium nitroprusside enhanced its effect in mesentery + pancreas only. In conclusion, vasodilator prostanoids, but not nitric oxide, counterregulate angiotensin II-mediated vasoconstriction in vivo.

Keywords: Angiotensin II; Prostanoid; Prostaglandin; Nitric oxide (NO); (Rat)

1. Introduction

Angiotensin II induces vasoconstriction via stimulation of angiotensin AT_1 receptors. Simultaneous vasodilation via stimulation of the angiotensin AT_2 receptor (Carey et al., 2000) may counteract this effect, although not all studies agree on this matter (Schuijt et al., 1999; Tanaka et al., 1999). In addition, vasodilator factors, such as nitric oxide (NO) and vasodilator prostanoids, may oppose the vasoconstrictor effects of angiotensin II. These factors are either released in response to vasoconstriction or via stimulation of angiotensin AT_1 and/or angiotensin AT_2 receptors on endothelial and vascular smooth muscle cells (Deng et al., 1996; Hennington et al., 1998; Jaiswal et al., 1993; Siragy et al., 1999; Toda and Miyazaki, 1981).

In support of this concept, NO-synthase (NOS) inhibition enhanced angiotensin II-induced vasoconstriction systemically as well as in the carotid artery and kidney (Boulanger et al., 1995; Champion et al., 1998). However, studies with NOS inhibitors have to be interpreted with care, because of the persistent rise in blood pressure which occurs during NOS inhibition. Indeed, an enhanced effect of angiotensin II was not observed in the kidney when NO was normalised by application of a NO donor during renal NOS inhibition (Aki et al., 1997; Ichihara et al., 1998; Parekh et al., 1996). Furthermore, angiotensin II infusion is accompanied by a rise in the levels of prostacyclin and/or prostaglandin E_2 in blood plasma (Toda and Miyazaki, 1981) and interstitial fluid (Darimont et al., 1994; Siragy et al., 1999). Receptor-mediated release of such vasodilator prostanoids may explain why angiotensin II induces vasodilation in cerebral (Haberl et al., 1990) and mesenteric (Chu and Beilin, 1993) arteries. However, angiotensin II also stimulates the release of vasoconstrictor prostanoids (e.g. thromboxane A_2 (Lin and Nasjletti, 1991)). Moreover, the cyclooxygenase inhibitor indomethacin attenuated the blood pressure lowering effects of both captopril and losartan, suggesting that blockade of angiotensin II synthesis or angiotensin AT_1 receptors is also accompanied by release of vasodilator prostanoids (Conlin et al., 2000).

In view of these discrepancies, it was the aim of the present study to investigate the role of NO and cyclooxygenase products in the angiotensin II-induced systemic and regional hemodynamic effects in vivo, using the radiolabelled microsphere method (Schuijt et al., 1999). By mea-

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suring radioactivity of microspheres trapped in end-arterioles detailed information on systemic hemodynamics as well as regional hemodynamics in all organs can be obtained, thereby extending previous studies on this issue that were either limited to the systemic hemodynamic effects of angiotensin II, or its effect in one particular bed.

2. Materials and methods

2.1. Instrumentation and hemodynamic measurements

All experiments were performed in accordance with the "Guiding principles in the care and use of animals" approved by the American Physiological Society and under the regulation of the Animal Care Committee of the Erasmus University Rotterdam, Rotterdam, The Netherlands. Experiments were carried out in male Wistar rats (body weight: 364 ± 7 g, mean $\pm$ S.E.M.; $n = 25$) obtained from Harlan, Zeist, The Netherlands.

Animals were anaesthetised with an intraperitoneal injection of sodium pentobarbital (60 mg/kg). To maintain an adequate depth of anaesthesia, intravenous bolus injections of sodium pentobarbital (5–10 mg/kg) were administered via the right external jugular vein every 15 min during the stabilisation period. A catheter was placed in the trachea for intermittent positive pressure ventilation with a mixture of oxygen and air, using a respiratory pump (Small Animal Ventilator, Harvard Apparatus, Natick, MA, USA). The ventilatory rate was adjusted to keep arterial blood gases within the physiological range. Blood pressure and heart rate were recorded with a pressure transducer (Combitrans Disposable Pressure Transducer, Braun, Melsungen, Germany) in the left femoral artery. Radioactive microspheres were injected into the left ventricle via a catheter in the right carotid artery. Drugs were administered via the right external jugular vein. The right femoral artery was cannulated to allow the withdrawal of reference blood samples.

After a one-hour stabilisation period following completion of instrumentation, the animals were given a 30-min infusion of the cyclooxygenase inhibitor indomethacin (5 mg/kg; $n = 8$), the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg; $n = 8$) followed by a continuous infusion of sodium nitroprusside at a rate required (approximately 4 μg/kg/min) to restore mean arterial blood pressure to baseline, or vehicle (saline, 0.1 ml/min; $n = 9$).

Next, three consecutive 10-min infusions of angiotensin II (100, 300 and 1000 ng/kg/min) were given to each animal. Prior to the angiotensin II infusions (baseline) and at the end of each angiotensin II infusion, when a steady state had been reached, hemodynamic parameters were measured and the distribution of aortic blood flow was determined by injecting 15.5 ± 0.1 (mean $\pm$ S.D.) μm diameter microspheres labelled with ^{141}Ce, ^{103}Ru, ^{95}Nb or ^{113}Sn (NEN Dupont, Boston, MA, USA). For each measurement about 200,000 microspheres, suspended in 0.2 ml saline and labelled with one of the isotopes, were mixed and injected into the left ventricle over a 15-s period. Following each injection, the catheter was thoroughly flushed with 0.5 ml saline. Starting 10 s before microsphere injection and lasting 70 s, an arterial reference blood sample was drawn from the right femoral artery at a constant rate of 0.5 ml/min, using a withdrawal pump (Model 55, Harvard Apparatus). At the end of the experiment the animal was sacrificed with an overdose of pentobarbital and all tissues were removed, weighed and put into vials. The radioactivity in the reference blood samples and the tissues was counted for 5 min in a γ-scintillation counter (Packard, Minaxi Auto-Gamma 5000 series, Downers Grove, IL, USA), using suitable windows discriminating the different isotopes. Lungs and heart were not evaluated, because the amount of radioactivity in the lungs mainly represents accumulation of microspheres that have bypassed the peripheral vascular beds (Saxena et al., 1980), whereas radioactivity in the heart represents both coronary flow and microspheres entrapped in the trabeculae during injection into the left ventricle. The latter may lead to falsely high values of coronary flow (Idvall et al., 1979), but will only marginally affect cardiac output (the sum of all tissue radioactivities) since it accounts for less than 2% of total body radioactivity.

2.2. Drugs

Indomethacin, L-NAME and sodium nitroprusside were obtained from Sigma (St. Louis, MO, USA) and angiotensin II was from Bachem Feinchemikalien (Bubendorf, Switzerland). Indomethacin (4 mg/ml) was dissolved in phosphate buffered saline (140 mM NaCl, 2.6 mM KCl, 1.4 mM KH_2PO_4 and 8.2 mM Na_2HPO_4) containing 7 mM NaOH and titrated to pH 7.4 with 6 M HCl. L-NAME (27 mg/ml), sodium nitroprusside (40 μg/ml) and angiotensin II (0.33, 0.99 and 3.30 μg/ml) were dissolved in saline.

2.3. Data presentation and statistical analysis

Data were processed as described previously (Saxena et al., 1980). Cardiac output and regional blood flow were calculated as follows:

$$\text{cardiac output} = \frac{\text{amount of radioactivity injected} \times \text{withdrawal rate of arterial blood sample}}{\text{radioactivity of arterial blood sample}}$$

$$\text{regional blood flow} = \frac{\text{tissue radioactivity} \times \text{cardiac output}}{\text{amount of radioactivity injected}}$$

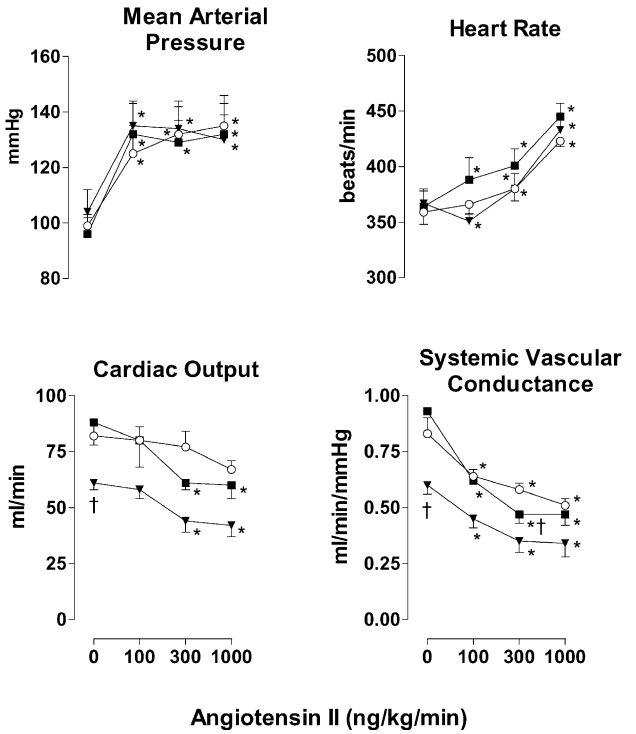

Fig. 1. Effects of 10-min intravenous infusions of angiotensin II on systemic hemodynamic parameters in rats pretreated with vehicle (0.1 ml/min; $n = 9$; open circles), indomethacin (5 mg/kg; $n = 8$; closed squares) or L-NAME (10 mg/kg; followed by a continuous infusion of sodium nitroprusside to restore mean arterial blood pressure to pre-L-NAME level; $n = 8$; closed triangles). Values are mean ± S.E.M. $^{*}P < 0.05$ vs. baseline, $\dagger P < 0.05$ vs. vehicle.

Systemic and regional vascular conductances (i.e. cardiac output and regional blood flow corrected for mean arterial blood pressure) were calculated to quantify the vasoconstrictor effects of angiotensin II with or without inhibition of NOS or cyclooxygenase.

All data are presented as mean ± S.E.M. Duncan's new multiple range test was used to test differences from baseline, once a two-way analysis of variance (ANOVA) had revealed that differences existed between the consecutive infusions. Student's unpaired *t*-test was used to evaluate the effects of the inhibitors, once two-way repeated measures ANOVA followed by Bonferroni's correction had revealed differences between the groups. Statistical significance was accepted at $P < 0.05$ (two-tailed).

3. Results

3.1. Systemic hemodynamic effects (Fig. 1)

Mean arterial blood pressure, cardiac output, heart rate and systemic vascular conductance were not affected by indomethacin. L-NAME + sodium nitroprusside reduced cardiac output and systemic vascular conductance by 26% and 28%, respectively ($P < 0.05$), but did not affect mean arterial blood pressure and heart rate. Angiotensin II increased mean arterial blood pressure and heart rate by maximally 35 ± 7% and 18 ± 3%, respectively, and decreased systemic vascular conductance by maximally 36 ± 7%. Cardiac output was not affected by angiotensin II. Neither indomethacin nor L-NAME + sodium nitroprusside affected any of the angiotensin II-induced systemic hemodynamic effects on mean arterial blood pressure, heart rate or cardiac output. Indomethacin, but not L-NAME + sodium nitroprusside, augmented the angiotensin II-induced decrease in systemic vascular conductance.

3.2. Regional hemodynamic effects

Basal blood flow values in adrenal, brain, gastrointestinal tract, kidney, liver, mesentery + pancreas, skeletal muscle, skin and spleen were 3.36 ± 0.70, 0.76 ± 0.05, 1.27 ± 0.13, 4.52 ± 0.36, 0.23 ± 0.04, 0.23 ± 0.03, 0.06 ± 0.01, 0.10 ± 0.01 and 2.3 ± 0.37 ml/min per g, respectively. L-NAME + sodium nitroprusside reduced renal and brain vascular conductance and increased vascular conductance in mesentery + pancreas. No significant changes were observed in the vascular conductance of other organs after L-NAME + sodium nitroprusside, nor did indomethacin alter the vascular conductances in any of the organs that were studied (Fig. 2). Angiotensin II decreased vascular conductance in gastrointestinal tract, kidney, skeletal muscle, skin, mesentery + pancreas, spleen and adrenal by maximally 37 ± 8%, 56 ± 5%, 26 ± 7%, 55 ± 7%, 28 ± 9%, 54 ± 6% and 33 ± 11%, respectively (Fig. 3). No effects of angiotensin II were observed on vascular conductance in brain and liver, which is most likely due to autoregulatory compensatory mechanisms in these organs. The effects of angiotensin II were augmented by indomethacin in all organs, although the differences were

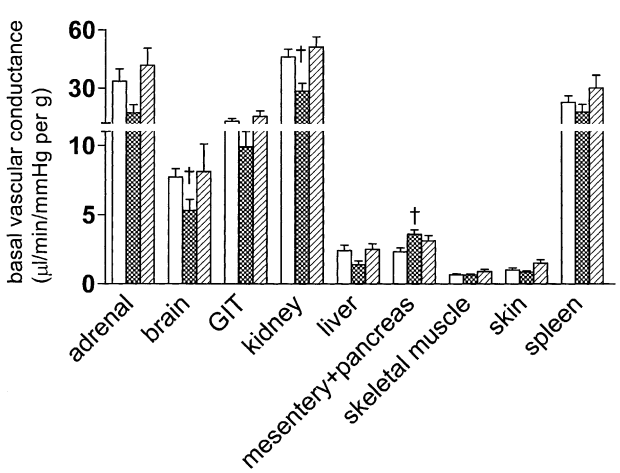

Fig. 2. Effects of pretreatment with vehicle (0.1 ml/min; $n = 9$; open bar), L-NAME (10 mg/kg; followed by a continuous infusion of sodium nitroprusside to restore mean arterial blood pressure to pre-blockade level; $n = 8$; dotted bar), or indomethacin (5 mg/kg; $n = 8$; hatched bar) on basal regional vascular conductance in rats. Values are mean ± S.E.M. GIT: gastrointestinal tract. $\dagger P < 0.05$ vs. vehicle.

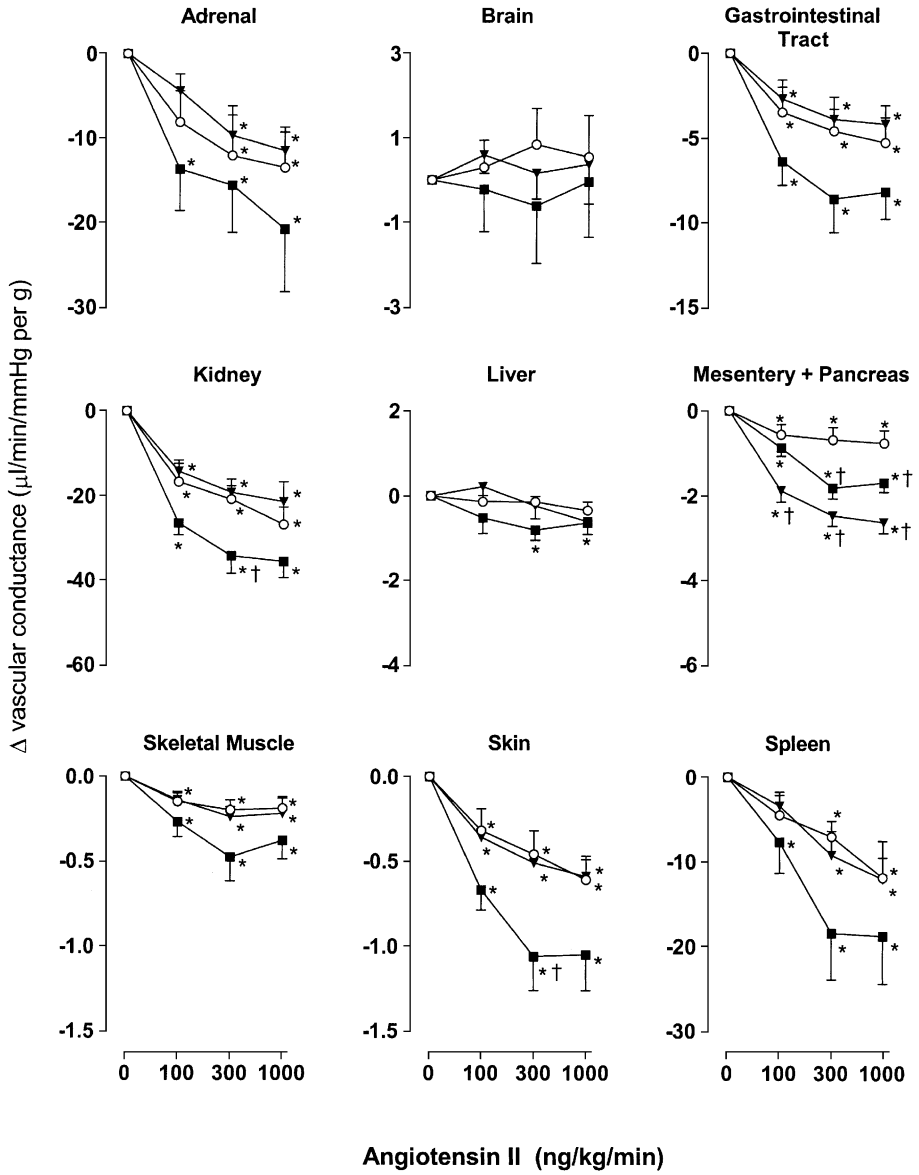

Fig. 3. Changes in regional vascular conductance during 10-min intravenous infusions of angiotensin II in rats, pretreated with vehicle (0.1 ml/min; $n = 9$; open circles), indomethacin (5 mg/kg; $n = 8$; closed squares) or L-NAME (10 mg/kg; followed by a continuous infusion of sodium nitroprusside to restore mean arterial blood pressure to pre-blockade level; $n = 8$; closed triangles). Values are mean ± S.E.M. $^{*}P < 0.05$ vs. baseline, †$P < 0.05$ vs. vehicle.

significant in skin, kidney and mesentery + pancreas only. L-NAME + sodium nitroprusside did not augment the angiotensin II-induced effects in any of the organs studied, with the exception of the mesentery + pancreas.

4. Discussion

The present study demonstrates that vasodilator prostanoids, but not NO, counterregulate the acutely induced systemic and regional vasoconstrictor effects of angiotensin II in vivo. The absence of NO-mediated counterregulatory effects in virtually all organs is in full agreement with previous studies in the kidney, where endogenous NO also did not counteract the vasoconstrictor effects of angiotensin II (Aki et al., 1997; Ichihara et al., 1998; Parekh et al., 1996). In these renal studies, like in our in vivo study, NO levels were normalised during NOS inhibition via application of sodium nitroprusside or *S*-nitroso-*N*-acetylpenicillamine. The dose of sodium nitroprusside administered during L-NAME application in our study was high enough to restore mean arterial blood pressure to preblockade level, although it did not restore cardiac output and vascular conductance in every vascular bed. The reduction in cardiac output might either be the consequence of the increase in systemic vascular resistance or it

may reflect NO saturation in the heart and a subsequent decrease in cardiac inotropy (Prendergast et al., 1997; Sigmon and Beierwaltes, 1993; Tom et al., 2001).

NO normalisation was not applied by Champion et al. (1998), and this may explain why these authors did observe an enhanced effect of angiotensin II in the renal vascular bed with NOS inhibition. Furthermore, our data do not argue against the concept that the renal vasoconstriction observed during NO inhibition, particularly during high renin states, is the consequence of unopposed angiotensin II-mediated effects (Sigmon and Beierwaltes, 1993; Sigmon et al., 1994). They merely demonstrate that angiotensin II infusion per se does not mediate NO release, neither directly (via angiotensin receptors) nor indirectly (i.e. as a consequence of shear stress).

The absence of NO-mediated effects during angiotensin II infusion opposes previous reports on angiotensin II-induced NO release via endothelial angiotensin AT_1 or angiotensin AT_2 receptors (Boulanger et al., 1995; Champion et al., 1998; Siragy et al., 1999). In an earlier study, using the same approach (angiotensin II infusions and regional blood flow measurements with microspheres), we were also unable to demonstrate angiotensin AT_2 receptor mediated hypotensive effects (Schuijt et al., 1999) and similar in vivo data were obtained by others (Champion et al., 1998; Touyz et al., 1999). Moreover, the initial reports on angiotensin AT_2 receptor-mediated vasorelaxation in angiotensin AT_2 receptor knockout mice (Hein et al., 1995; Ichiki et al., 1995) were disputed by subsequent studies (Saavedra et al., 2001; Tanaka et al., 1999) demonstrating that the enhanced angiotensin II-mediated vasoconstriction in angiotensin AT_2 receptor knockout mice is due to upregulation of angiotensin AT_1 receptors rather than the absence of vasodilator angiotensin AT_2 receptors. Furthermore, removal of the endothelium as well as preincubation with L-NAME did not affect the angiotensin AT_1 receptor-mediated contractions in human subcutaneous arteries (Garcha et al., 1999), thereby supporting our data on the absence of endothelial angiotensin AT_1 receptor-mediated NO release.

Prostacyclin is known to counteract angiotensin II-induced vasoconstriction (Parisi and Walsh, 1989). Moreover, stimulation of both angiotensin AT_1 and angiotensin AT_2 receptors on endothelial and vascular smooth muscle cells has been reported to result in the release of vasodilator and vasoconstrictor prostanoids, including prostacyclin, prostaglandin E_2 (Jaiswal et al., 1993) and thromboxane A_2 (Lin and Nasjletti, 1991). A recent study in human isolated subcutaneous resistance arteries (Garcha et al., 1999), however, does not support the latter concept, suggesting that if prostanoids are released during angiotensin II infusion, this is due to the rise in blood pressure. Based on the present study, it cannot be concluded whether the release of prostanoids is a direct consequence of angiotensin receptor stimulation or occurs in response to the regional and systemic vasoconstrictor effects of angiotensin II (i.e. occurs as a consequence of shear stress). Although the angiotensin II-induced release of prostanoids differs depending on the vascular bed, resulting in both vasodilator (Chu and Beilin, 1993; Haberl et al., 1990) and vasoconstrictor (Lin and Nasjletti, 1991) effects in vitro, our data indicate a predominance of vasodilator prostanoids during angiotensin II infusion in virtually all vascular beds. Our study, using the radiolabelled microsphere method, is the first to investigate the role of NO and prostanoids in all regional vascular beds at the same time.

The dose of indomethacin used in the present study has been reported to attenuate the responses mediated by the prostanoid precursor, arachidonic acid (Hui and Falardeau, 1990; Quintana et al., 1983). Indomethacin did not alter systemic and regional hemodynamics, thereby indicating that, normally, prostanoids do not play a major regulatory hemodynamic role. Furthermore, the rise in blood pressure occurring during L-NAME administration in this study confirms its NOS-inhibitory effect at the dose applied.

In conclusion, vasodilator prostanoids are more important than NO in counterregulating the angiotensin II-mediated systemic and regional hemodynamic effects in Wistar rats.

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