Effect of Blocking Angiotensin II Receptor Subtype on Rat Sympathetic Nerve Function

Pancras C. Wong, Roberta Bernard, and Pieter B.M.W.M. Timmermans

This study examined effects of nonpeptide angiotensin II (Ang II) receptor subtype antagonists on the interaction of sympathetic function and Ang II in pithed rats. Effects of spinal cord stimulation (0.5–4 Hz) and norepinephrine (0.3–3 /g/kg i.v.) on mean arterial pressure (recorded with a carotid arterial catheter), cardiac output (measured with an electromagnetic flowmeter and flow probe around the thoracic ascending aorta), total peripheral resistance, and heart rate were determined. The subtype 1–selective Ang II receptor antagonist losartan (previously known as DuP 753) at 10 mg/kg i.v. blocked the hemodynamic responses to Ang II at 1 /g/kg i.v. It inhibited mean arterial pressure and total peripheral resistance responses but not cardiac output and heart rate responses to spinal cord stimulation. In contrast, it reduced mean arterial pressure and cardiac output responses but not total peripheral resistance and heart rate responses to intravenous norepinephrine. Given at 100 mg/kg i.v., the subtype 2–selective receptor antagonist PD123177 did not reduce hemodynamic responses to intravenous Ang II, spinal cord stimulation, and intravenous norepinephrine. These results suggest that endogenous Ang II facilitates the release of norepinephrine from sympathetic nerve terminals in the vasculature of pithed rats. Similar to the Ang II receptor in vascular smooth muscle, the prejunctional Ang II receptor in pithed rats appears to be of subtype 1. (Hypertension 1992;19:663–667)

KEY WORDS • angiotensin receptors • antihypertensive therapy • renin-angiotensin system • sympathetic nervous system

It is well known that exogenous angiotensin II (Ang II) enhances vasoconstrictor and vascular contractile responses to sympathetic nerve stimulation.1,2 The adrenergic potentiation by Ang II has been commonly attributed to increased release of norepinephrine through the activation of prejunctional Ang II receptors, inhibition of the uptake of norepinephrine from sympathetic nerve terminals, and increased vascular responsiveness to norepinephrine.1,2 By blockade of the influence of the renin-angiotensin system with angiotensin converting enzyme inhibitors or Ang II receptor antagonists, the adrenergic facilitating effect of endogenous Ang II has also been shown.3–8

Recently, we as well as others demonstrated that Ang II receptors may be subdivided pharmacologically into two subtypes, i.e., subtype 1 (AT1) and subtype 2 (AT2) (for review, see References 9 and 10). In anesthetized dogs, the AT1-selective receptor antagonist losartan (previously known as DuP 753, 2-n-butyl-4-chloro-5-(hydroxymethyl)-1-[(2'-(1/-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole, potassium salt) blocked the renal vasoconstrictor response to renal nerve stimulation but not to norepinephrine, whereas the AT2-selective receptor antagonist PD123177 (1-[(4-amino-3-methylphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid) was ineffective.8 This result suggests that endogenous Ang II enhances renal adrenergic function at the prejunctional site through the activation of AT1 receptors. To further substantiate this finding, we examined the effects of losartan and PD123177 on hemodynamic responses to sympathetic nerve stimulation in the pithed rat, a model commonly used to study the adrenergic potentiation of endogenous Ang II.

Methods

The pithed rat model, described by Kaufman and Vollmer,9 was used in this study with some modifications.6 Male CD Sprague-Dawley rats (250–400 g; Charles River Laboratories, Inc., Kingston, N.Y.) were anesthetized with hexobarbital (150 mg/kg i.p.), adrenalectomized, pithed, and artificially respirated. Atropine (2 mg/kg i.p.) was given, and both vagus nerves were cut to prevent any parasympathetic effect. The carotid artery and jugular vein were cannulated for arterial pressure measurement with a pressure transducer (P23ID, Gould Inc., Oxnard, Calif.) and intravenous injection of drugs, respectively. Tubocurarine (1 mg/kg i.v.) was given to prevent voluntary muscle activity. A calibrated electromagnetic flow probe connecting to a flowmeter (Carolina Medical Electronics, Inc., King, N.C.) was placed on the thoracic ascending aorta to measure cardiac output (CO) (excluding coronary blood flow).

Mean arterial pressure (MAP) was determined as the sum of diastolic blood pressure and one third of the pulse pressure. CO was expressed as milliliters per minute per kilogram of body weight. Total peripheral resistance (TPR) was calculated by dividing MAP by CO and then multiplying by 100. Blood pressure and
TABLE 1. Baseline Hemodynamics and Responses to Angiotensin II Before and After Losartan or PD123177

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Losartan Before</th>
<th>Losartan After</th>
<th>PD123177 Before</th>
<th>PD123177 After</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>45±2</td>
<td>33±1*</td>
<td>49±3</td>
<td>48±1</td>
</tr>
<tr>
<td>CO (ml/min/kg)</td>
<td>115±4</td>
<td>88±5*</td>
<td>124±7</td>
<td>119±6</td>
</tr>
<tr>
<td>TPR (units)</td>
<td>39±1</td>
<td>38±2</td>
<td>40±2</td>
<td>41±2</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>325±7</td>
<td>303±5*</td>
<td>343±8</td>
<td>332±12*</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=10 per group for baseline hemodynamic data (pooled data from groups treated with spinal cord stimulation and norepinephrine) and n=5 per group for angiotensin II (Ang II) response data. Ang II was given at 1 μg/kg i.v., losartan at 10 mg/kg i.v., and PD123177 at 100 mg/kg i.v. MAP, mean arterial pressure; CO, cardiac output; TPR, total peripheral resistance; HR, heart rate; bpm, beats per minute.

In the first group of experiments, the spinal cord was inserted under the skin of the hind limb as the anode. Fifteen minutes later, hemodynamic responses to spinal cord stimulation were again determined.

Results

As shown in Table 1, losartan at 10 mg/kg i.v. lowered baseline MAP, which was due to a decrease in baseline CO. PD123177 at 100 mg/kg i.v. did not alter baseline MAP, CO, or TPR. Both compounds slightly reduced heart rate (HR). Saline vehicle (n=10) did not alter baseline MAP, CO, or TPR (data not shown) but slightly decreased HR from 292±8 to 276±8 beats per minute (p<0.05). Thus, the slight reduction of baseline HR by losartan and PD123177 may not be pharmacologically relevant. Ang II at 1 μg/kg i.v. increased MAP, CO, TPR, and HR (Table 1). These responses to Ang II were blocked by losartan but not by PD123177.

As shown in Figure 1, electrical stimulation of the spinal cord caused a frequency-dependent increase in MAP, which is due to increases in CO and TPR. Losartan at 10 mg/kg i.v. reduced the pressor responses to spinal cord stimulation by a reduction of the TPR but not the CO component. The HR response was not changed significantly by losartan. On the contrary, PD123177 at 100 mg/kg i.v. did not alter the hemodynamic responses to spinal cord stimulation. Saline vehicle did not change the hemodynamic responses to spinal cord stimulation (data not shown).

Norepinephrine caused a dose-dependent increase in MAP, CO, TPR, and HR (Figure 2). Losartan at 10 mg/kg i.v. reduced the MAP and CO responses to norepinephrine significantly but not the TPR and HR responses. The tendency for a reduction of the TPR response to norepinephrine at 0.3 μg/kg i.v. after losartan was not significant. PD123177 at 100 mg/kg i.v. increased the MAP and TPR responses to norepinephrine significantly (Figure 2). Saline vehicle did not change the hemodynamic responses to norepinephrine (data not shown).

Discussion

Two distinct high-affinity binding sites for Ang II have recently been identified in various tissues by radioligand receptor binding and autoradiographic studies (for review, see References 9 and 10). The Ang II binding sites that have high affinity for losartan or its analogues are classified as AT₁, whereas those that have high affinity for PD123177, PD123319, CGP42112A, and pNH₂-[Phe]Ang II are defined as AT₂ sites. Most of the known effects of Ang II, including vasoconstriction, adrenal aldosterone and catecholamine secretion, and water drinking, were blocked by the AT₁ receptor antagonist losartan (or its analogues), whereas the AT₂ receptor antagonist PD123177 (or its analogues) was ineffective. This study shows that losartan at 10 mg/kg i.v. almost completely abolished the hemodynamic responses to Ang II, whereas PD123177 at 100 mg/kg i.v. did not alter these effects of Ang II in pithed rats. This confirms previous findings that the vasoconstrictor effect of Ang II in rats is mediated by the AT₁ receptor.

Losartan but not PD123177 decreased baseline MAP in the pithed rat, which is mainly due to a reduction in CO. This is consistent with previous studies showing that the hypotensive effect of saralasin, a peptide Ang II receptor antagonist, in the pithed rat was due to a reduction of CO without alteration of TPR. Apparently, in the absence of tonic sympathetic discharge, Ang II does not contribute to TPR in the pithed rat. The positive chronotropic effect of Ang II (see Table 1) may not be related to the maintenance of CO, because...
the high peak plasma Ang II level after bolus intravenous Ang II may not be physiologically relevant. In fact, in captopril-treated pithed rats, intravenous infusion of Ang II at a dose that reversed the reduced CO to precaptopril levels (i.e., presumably returned the depressed plasma Ang II level to control level) did not alter HR.5 As Ang II does not have a direct positive cardiac contractile effect in rats,16 it is likely that Ang II maintains CO by inducing venous constriction5,17 through the activation of the AT₁ receptors.

Our present study confirms previous findings5,6 that stimulation of the sympathetic neurons in the pithed rat raises blood pressure by increasing CO and TPR. Blockade of the AT₁ receptors with losartan significantly attenuated the pressor response to sympathetic nerve stimulation. This is solely due to a reduction of the rise in TPR. CO and HR responses to sympathetic nerve stimulation were not significantly affected. Similar results were also reported in rats with the angiotensin converting enzyme inhibitor captopril and the peptide Ang II receptor antagonist saralasin.4–6 One possible explanation suggested by Kaufman and Vollmer5 is that circulating Ang II gains ready access to the vascular but not to the cardiac sympathetic synapses in the pithed rat. However, our previous study with a monoclonal Ang II antibody suggests that vascular-formed Ang II but not circulating Ang II enhances the sympathetic vascular response in the pithed rat.6 An alternate explanation is that the cardiac prejunctional Ang II receptor in rats may be absent or not function in vivo.

Norepinephrine injected intravenously in the pithed rat caused dose-dependent increases in MAP. Similar to sympathetic nerve stimulation, the pressor response to norepinephrine was accounted for increases in CO and TPR. Inhibition of the AT₁ receptors with losartan also reduced the pressor response to norepinephrine. However, unlike the case with sympathetic nerve stimulation, the reduction of the CO component but not the TPR component contributed to the decrease in pressor response to norepinephrine. The mechanisms accounting for the reduction in CO increases to norepinephrine by losartan are not clear. However, it is not likely that losartan affects the cardiac response to β-adrenergic receptor stimulation, because it did not alter the tachycardiac response to isoproterenol18 and the cardiac response to sympathetic nerve stimulation as shown in this study. Previous studies reported that captopril and saralasin reduced the pressor effect of norepinephrine by decreasing the TPR but not the CO response in the pithed rat.6,5 The reasons for the discrepant results

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**FIGURE 1.** Line plots show mean arterial pressure, cardiac output, total peripheral resistance, and heart rate responses to spinal cord stimulation in pithed rats before and after losartan or PD123177. Values represent mean±SEM; n=5 per group; p<0.05 compared with corresponding pretreatment control (analysis of variance).
between these studies are not obvious but may be due to other actions of captopril and saralasin. For instance, unlike saralasin and captopril, losartan does not possess the Ang II agonistic and bradykinin potentiating effects, respectively. Interestingly, PD123177 enhanced the pressor response to norepinephrine by increasing the TPR without altering CO. Apparently, this is not due to a nonselective increased responsiveness of the vasculature to vasoconstriction, as the vasoconstrictor effects of Ang II and sympathetic nerve stimulation were not changed by PD123177. The pharmacological significance of this enhancement by PD123177 is not known. Perhaps additional studies using more selective α-adrenergic receptor agonists and different subtype Ang II receptor antagonists may clarify these findings.

In summary, as the TPR response elicited by sympathetic nerve stimulation but not the TPR response induced by intravenous norepinephrine was reduced by losartan, a prejunctional rather than a postjunctional inhibition may account for the observed reduced responses to sympathetic nerve stimulation in the pithed rat. Furthermore, the prejunctional Ang II receptors in the vasculature of the pithed rat are AT1 receptors, which is consistent with our previous finding that the renal prejunctional Ang II receptors in anesthetized dogs are of the AT1 type.

References


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