Effects of Angiotensin Receptor Subtype Inhibitors on Plasma Angiotensin Clearance

Melchiore A. Vernace, Peter F. Mento, Mary E. Maita, Barry M. Wilkes

Abstract The aim of this study was to determine whether angiotensin receptor subtypes play a role in angiotensin clearance from plasma. Angiotensin metabolic clearance rate was measured in rats by the constant infusion method. Increasing doses of angiotensin II were infused for 15 minutes, and blood was sampled for angiotensin II. The type 1 angiotensin II receptor antagonist losartan decreased the apparent metabolic clearance rate by >50% at low-dose infusion, suggesting that type 1 angiotensin II receptors are involved in angiotensin II clearance from plasma. At higher angiotensin infusion rates, the metabolic clearance rate of angiotensin was unaffected. To dissect the contribution of renin-generated angiotensin, additional experiments were performed in nephrectomized rats. In anephric rats, angiotensin clearance was unaffected by type I angiotensin II receptor inhibition. In contrast, the type 2 angiotensin II receptor ligand PD123319 in intact rats caused a >50% increase in metabolic clearance rate of angiotensin at higher infusion rates (P<.05). In anephric rats, the type 2 angiotensin II receptor ligand alone or combined with type 1 receptor inhibition was without effect on the metabolic clearance rate or the T1/2 for angiotensin disappearance. These data argue against a role for type 1 or 2 angiotensin II receptors as clearance receptors. Increased clearance of angiotensin by type 2 receptor blockade in the presence but not the absence of kidneys suggests an alternative renal mechanism by which selective type 2 ligands may alter angiotensin effects.

Key Words • angiotensin II • renin-angiotensin system • radioimmunoassay • receptors, angiotensin • losartan

Recent nonpeptide ligands have been used to classify two types of angiotensin II receptors (AT1 and AT2). AT1 receptors are found in blood vessels and renal glomeruli; they mediate the hypertensive effects of angiotensin II (Ang II). AT1 receptors have also been shown to mediate catecholamine and aldosterone secretion and drinking responses to Ang II. AT2 receptors have been shown to exist in at least two different forms (AT2a and AT2b). AT2 receptors are found in adrenal, brain, and fetal tissues, but their function is not yet understood, although some studies suggest a role in renal function.

Recent studies of atrial natriuretic peptide have demonstrated the presence of a "clearance receptor" for this hormone. We hypothesized that one or more of the Ang II receptor subtypes may serve a similar role for clearing Ang II from plasma. We used specific ligands of AT receptor subtypes in rats to test the potential roles of AT1 and AT2 receptor subtypes in the metabolic clearance of Ang II from plasma.

Methods

Materials

All chemicals were of the purest commercial grade available. Angiotensin I (Ang I) and [Ile5]angiotensin II (Ang II) were purchased from Sigma Chemical Co. Ang II was purchased from El Du Pont de Nemours & Co, Inc/NEN Products. Losartan was a gift from DuPont Merck Pharmaceutical Co, and PD123319 ((S)-1-[(dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid) was a gift from Warner-Lambert Co.

Studies were performed in male Sprague-Dawley rats (357±3 g; n=43) given ad libitum access to standard Purina rat chow (Ralston Purina Co) (0.42% sodium ash content) and tap water. All protocols were in accordance with institutional guidelines for the care and handling of laboratory animals.

Angiotensin Metabolic Clearance Rate

Rats were anesthetized with Inactin (100 mg/kg IP), and catheters (PE50) were placed in the carotid artery (for blood pressure monitoring and blood sampling), jugular vein (Ang II infusions), and femoral vein (drug or vehicle infusions). Plasma from blood samples (1.3 mL) was frozen at -20°C until assay for Ang II and plasma renin activity (PRA). Packed cells were returned to the rats resuspended in 13.4% Ficoll. Ang II was infused at 0, 5, 14, 50, 144, and 556 ng/kg per minute for 15 minutes. In the steady state, the removal rate of Ang II is equal to the infusion rate plus the rate of endogenous production. A 100-fold range of infusion concentrations (5 to 556 ng/kg per minute) was studied to achieve plasma levels of Ang II that were at least 10-fold higher than endogenous levels to minimize the contribution of endogenous angiotensins. These conditions were met at the two highest doses for all groups of rats with intact kidneys. Anephric rats achieved >10-fold elevation of plasma angiotensin II levels at 14 ng/kg per minute. Therefore, all studies in anephric rats were performed at this dose only. Metabolic clearance rate (MCR) was calculated as

\[ \text{MCR} = \frac{\text{Infusion Rate of Ang II}}{\text{Plasma Ang II Concentration}} \]

where MCR is in milliliters per minute per kilogram body weight, infusion rate is in nanograms per kilogram per minute, and concentration is in nanograms per milliliter.
Analytical Measurements

PRA and plasma Ang II were measured as previously described. Immunoreactive Ang II was measured by direct radioimmunoassay (RIA) after extraction. Measurements of serial dilutions of plasma samples containing losartan and PD123319 were parallel to the standard curve for Ang II, indicating that there was no direct effect of either agent on the Ang II measurement. In preliminary studies, three plasma samples from rats infused with Ang II (144 ng/kg per minute) were studied after high-performance liquid chromatographic (HPLC) fractionation. Immunoreactive Ang II that coeluted with synthetic Ang II peptide accounted for 88±1% of total immunoreactive Ang II. Therefore, all subsequent experiments were performed with direct RIA.

Protocols

Studies in Intact Rats

After baseline blood sampling, either vehicle (saline), losartan (3 mg/kg bolus + 0.3 mg/kg per hour infusion), or PD123319 (3 mg/kg bolus + 3 mg/kg per hour infusion) was given. A second blood sample was taken before the Ang II infusion (5 ng/kg per minute) was started. After 15 minutes, a third blood sample was taken, and the infusion rate was increased successively (14, 50, 144, and 556 ng/kg per minute) with blood sampling at 15 minutes after each increment. The dose of losartan has been shown to blunt pressor responses to angiotensin II. Although it was not possible to titrate the dose of PD123319, we used an infusion dose three times that which has been shown to cause renal effects in dogs.

Studies in Nephrectomized Rats

Rats were bilaterally nephrectomized under pentobarbital anesthesia, and metabolic clearance rates were measured at 24 hours. Plasma Ang II was measured (0.1 ng Ang I/mL per hour) in these rats. After baseline blood sampling, Ang II was infused (14 ng/kg per minute) for 15 minutes and a second blood sample taken. In two other groups of rats (n=6), losartan (3 mg/kg) and PD123319 (3 mg/kg) were given in random order with blood sampling 15 minutes later. According to a crossover design, rats that had received losartan were then given PD123319 and vice versa, and after 15 minutes another blood sample was taken. The Ang II infusion was then discontinued, and blood samples taken at 1, 2, and 5 minutes after the infusion was stopped to measure the time course of the disappearance of Ang II from plasma.

Data Analysis

Data are presented as the mean±SEM. Differences between groups were tested by analysis of variance (ANOVA) followed by the Student-Newman-Keuls method or repeated-measures ANOVA for multiple comparisons. The null hypothesis was rejected when P<.05 (two-tailed).

Results

The administration of the AT1 receptor antagonist losartan lowered mean arterial pressure (MAP) from 118±5 to 87±8 mm Hg (P<.01). The reduction in MAP was associated with a greater than fivefold increase in plasma Ang II levels. In contrast, the AT2 receptor ligand PD123319 was without effect on MAP (baseline, 111±3 mm Hg; PD123319, 101±4 mm Hg; P=NS) or plasma Ang II. When high doses of Ang II were infused, MAP remained significantly lower in the losartan-treated rats compared with either controls or PD123319-treated rats (Fig 1). Losartan caused a threefold increase in PRA. The rise in PRA was not suppressed below baseline even with high-dose Ang II infusion (Table).

Table

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (n=8)</th>
<th>Losartan (n=8)</th>
<th>PD123319 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>15±4</td>
<td>23±6</td>
<td>36±6</td>
</tr>
<tr>
<td>Vehicle or drug</td>
<td>24±4</td>
<td>72±13*</td>
<td>43±6</td>
</tr>
<tr>
<td>Angiotensin II infusion</td>
<td></td>
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<tr>
<td>ng/kg per min</td>
<td></td>
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<tr>
<td>5</td>
<td>27±4</td>
<td>51±8</td>
<td>37±6</td>
</tr>
<tr>
<td>14</td>
<td>20±4</td>
<td>43±9</td>
<td>34±6</td>
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<tr>
<td>50</td>
<td>9±1*</td>
<td>32±4†</td>
<td>12±1‡</td>
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<tr>
<td>144</td>
<td>5±1†</td>
<td>24±3†</td>
<td>6±1‡</td>
</tr>
<tr>
<td>556</td>
<td>4±1†</td>
<td>19±2†</td>
<td>4±1†</td>
</tr>
</tbody>
</table>

Values are in nanograms angiotensin I per milliliter per hour.

*P<.01 vs baseline; †P<.05 vs vehicle or drug value; §P<.01 vs vehicle or drug value; ¶P<.001 vs vehicle or drug value.

The apparent MCRAngI was studied after incremental infusions of Ang II. Compared with untreated controls, losartan-treated rats achieved higher plasma Ang II levels at the low infusion rates (5, 14, and 50 ng/kg per minute, P<.05) but achieved levels comparable to controls at 144 and 556 ng/kg per minute (P=NS) (Fig 2). PD123319 was without effect on plasma Ang II levels except at the two highest doses, at which Ang II levels were significantly reduced compared with controls or losartan-treated rats (P<.05). When the MCRAngI was calculated from these data, there was an apparent reduction in losartan-treated rats at infusion rates of 5, 14, and 50 ng/kg per minute (P<.05), but there were no differences in MCRAngI at the higher infusion rates compared with untreated control rats (Fig 3). PD123319 was without effect on the apparent MCRAngI at the low-dose infusions (5, 14, and 50 ng/kg per minute), but the apparent MCRAngI was increased at the two highest doses (P<.05) (Fig 3).

To determine the effects of AT1 and AT2 receptor blockade on MCRAngI from plasma independent of...
CD

Infused Ang II (ng/kg/min)

10

1.0

0.1

Plasma Ang II (ng/ml)

10

1.0

0.1

30 100 1000

Infused Ang II (ng/kg/min)

10 100 1000

0.1

Plasma Ang II (ng/ml)

10

1.0

0.1

Fig. 2. Graph shows plasma angiotensin II (Ang II) levels during graded infusions of Ang II. Graded infusions of Ang II (5, 14, 50, 144, and 556 ng/kg per minute) were given to control rats (vehicle; n=8), rats treated with losartan (3 mg/kg bolus + 0.3 mg/kg per hour infusion; n=8), and rats treated with PD123319 (3 mg/kg bolus + 3 mg/kg per hour infusion; n=8). Compared with controls, losartan-treated rats achieved higher plasma Ang II levels at the low infusion rates (5, 14, and 50 ng/kg per minute) but showed no difference at higher Ang II infusion rates (144 and 556 ng/kg per minute). At higher Ang II infusion rates (144 and 556 ng/kg per minute), plasma Ang II levels were lower in PD123319-treated rats. SEM lies within data points. *P<.05.

Fig. 3. Graph shows the effects of angiotensin II (Ang II) receptor subtype inhibition on the metabolic clearance rates of Ang II (MCRAngII). MCRAngII was measured at a single infusion rate of Ang II (14 ng/kg per minute). MCRAngII was unaffected by either losartan (122±12 mL/min per kilogram, n=6) or PD123319 (197±40 mL/min per kilogram, n=6) compared with controls (118±31 mL/min per kilogram, n=7). After these measurements with either an AT1 or AT2 receptor inhibitor, additional experiments were performed in these rats with combined AT1 and AT2 receptor inhibition. After the combined administration of both AT1 and AT2 receptor inhibitors, the Ang II infusion was stopped and the time course for disappearance of Ang II was measured. As shown in Fig 4, the T1/2 of Ang II was unaffected by combined AT1 and AT2 inhibition compared with untreated controls (AT1+AT2 inhibition, 0.67±0.05 minutes, n=12; P=NS vs control). Control (•): A indicates saline; B, saline. Losartan (a): A indicates losartan; B, PD123319. PD123319 (A): A indicates PD123319; B, losartan.

Fig. 4. Graph shows the effects of combined angiotensin II (Ang II) receptor subtype inhibition on the disappearance of Ang II peptide from plasma in nephrectomized rats. Twenty-four hours after bilateral nephrectomy, rats were studied with a continuous infusion of Ang II (14 ng/kg per minute). After 15 minutes of Ang II infusion, a blood sample was taken, and in control rats, two additional blood samples were taken at 15-minute intervals. In two other groups of rats (n=6), losartan (3 mg/kg) and PD123319 (3 mg/kg) were both given in randomized order with blood sampling 15 minutes after each. T1/2 for disappearance of Ang II was measured after the cessation of the Ang II infusion. Washout of Ang II was 0.63±0.07 minutes in control rats. T1/2 of Ang II was unaffected by combined AT1 and AT2 inhibition (AT1+AT2 inhibition, 0.67±0.05; n=12; P=NS vs control). Control (•): A indicates saline; B, saline. Losartan (a): A indicates losartan; B, PD123319. PD123319 (A): A indicates PD123319; B, losartan.

Discussion

The concentration of Ang II in plasma is dependent on the rates of both production and removal of the Ang II peptide. Plasma Ang II is derived by the action of renin on angiotensinogen in plasma or by the local generation and release of Ang II peptide from cells that contain specific mRNAs for renin, angiotensinogen, and angiotensin-converting enzyme. The latter mechanism is supported by recent studies using molecular techniques8 and by studies in our laboratory that demonstrated the presence of plasma angiotensins in the plasma of anephric patients maintained on hemodialysis for prolonged periods of time.9

Although a great deal is known about the factors that regulate the formation of Ang II, much less is known about the mechanisms by which Ang II is removed from plasma. Recent studies of atrial natriuretic peptide have led to the concept that peptidic hormones may be removed from plasma by specific receptors called "clearance receptors."6 We hypothesized that one or more of the Ang II receptor subtypes may serve a similar role for removing Ang II from plasma.

renal Ang II production, additional studies were performed in nephrectomized rats. MCRAngII was measured at a single infusion rate of Ang II (14 ng/kg per minute). MCRAngII was unaffected by either losartan (122±12 mL/min per kilogram, n=6) or PD123319 (197±40 mL/min per kilogram, n=6) compared with controls (118±31 mL/min per kilogram, n=7). After these measurements with either an AT1 or AT2 receptor inhibitor, additional experiments were performed in these rats with combined AT1 and AT2 receptor inhibition. After the combined administration of both AT1 and AT2 receptor inhibitors, the Ang II infusion was stopped and the time course for disappearance of Ang II was measured. As shown in Fig 4, the T1/2 of Ang II was unaffected by combined AT1 and AT2 inhibition compared with untreated controls (AT1+AT2 inhibition, 0.67±0.05 minutes, n=12, versus control, 0.63±0.07 minutes, n=7; P=NS).
In the present study, losartan decreased the apparent MCR\textsubscript{AngII} by >50% at a low rate of Ang II infusion in rats with intact kidneys but not at higher Ang II infusions. PRA was stimulated by losartan, and high-dose Ang II infusions did not suppress PRA below baseline values (Table). Two explanations were considered. Endogenously produced Ang II could have contributed significantly to the measured levels of plasma Ang II at low-dose infusion, or clearance receptors that removed a large percentage of Ang II at low-level infusions may have become saturated at high-dose Ang II infusions, masking the potential role of these receptors at physiological concentrations of Ang II. To differentiate between these possibilities, other studies were performed in nephrectomized rats. In these rats, Ang II infusion resulted in lower plasma Ang II (higher MCR\textsubscript{AngII}) compared with intact rats. AT\textsubscript{1} and AT\textsubscript{2} inhibition alone or in combination had no effect on MCR\textsubscript{AngII}.

AT\textsubscript{2} receptor inhibition in rats with intact kidneys caused a >50% increase in the MCR\textsubscript{AngII} (P<.05). This finding was unexpected and was probably mediated by a renal mechanism, since AT\textsubscript{2} inhibition in anephric rats was without effect on MCR\textsubscript{AngII}.

The values obtained for MCR\textsubscript{AngII} and T\textsubscript{1/2} in our studies in rats with intact kidneys agree closely with values reported for humans and other mammals.10-12 MCR\textsubscript{AngII} in anephric rats is significantly higher, suggesting that the net effect of the kidneys to release PRA to form Ang II in the circulation is greater than the effect of the kidneys on Ang II degradation.

The Ang II levels in the present experiments were measured with direct RIA, which may slightly overestimate the values of immunoreactive Ang II, since Ang III, Ang II-(3-8) hexapeptide, and Ang II-(4-8) pentapeptide cross-react to various degrees with the antibody.13 Plasma samples in Ang II-infused rats that were analyzed after HPLC separation of angiotensin peptides had >88% of the immunoreactive material coelute with authentic Ang II peptide. Therefore, direct RIA of plasma samples gives a good estimate of MCR\textsubscript{AngII}.

Several studies have examined the mechanisms for angiotensin II degradation from the plasma. Bumpus et al14 studied the fate of radiolabeled Ang II and found that most tissues contained potent Ang II degradative capacity. Others confirmed these findings by comparing Ang II degradation in plasma and across vascular beds and concluded that plasma angiotensinases were much less important than tissue factors for removal of the peptide.15 Cleavage of Ang II at the amino terminus appears to be an important route of degradation,16,17 but the precise mechanisms by which tissues cleave Ang II has not been elucidated. The present experiments suggest that the mechanism is independent of AT\textsubscript{1} and AT\textsubscript{2} receptor subtypes and that these receptors do not function as clearance receptors. The surprising observation, however, was that AT\textsubscript{2} receptor inhibition in rats with intact kidneys caused a significant increase in the clearance of Ang II from plasma at high Ang II dose infusions. This interesting finding will require further investigation.

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References
9. Wilkes BM, Mento PF, Pearl AR, Hollander AM, Mossey RT, Vernace. Several studies have examined the mechanisms for angiotensin II degradation from the plasma. Bumpus et al14 studied the fate of radiolabeled Ang II and found that most tissues contained potent Ang II degradative capacity. Others confirmed these findings by comparing Ang II degradation in plasma and across vascular beds and concluded that plasma angiotensinases were much less important than tissue factors for removal of the peptide.15 Cleavage of Ang II at the amino terminus appears to be an important route of degradation,16,17 but the precise mechanisms by which tissues cleave Ang II has not been elucidated. The present experiments suggest that the mechanism is independent of AT\textsubscript{1} and AT\textsubscript{2} receptor subtypes and that these receptors do not function as clearance receptors. The surprising observation, however, was that AT\textsubscript{2} receptor inhibition in rats...
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