Angiotensin III Depressor Action in the Conscious Rabbit Is Blocked by Losartan but not PD 123319

Brian P. Rowe, Byron Dixon

Abstract—Vasodilator and vasodepressor properties of angiotensins have been reported, and mediation by prostaglandins or nitric oxide has been proposed. Other studies indicate that angiotensin AT2 receptors might mediate a depressor action, and the present study was designed to delineate and explore this possibility in a conscious rabbit model. Large intravenous boluses of angiotensin III (15 nmol/kg) produced a predictable pressor peak (82±4 mm Hg) followed by a depressor phase (20±3 mm Hg), whereas equipressor doses of angiotensin II were less effective at producing depressor responses. Angiotensin-(1–7) did not exert a depressor action, and the reduced potency of angiotensin IV (relative to angiotensin III) was similar for both the pressor and depressor phases (=100-fold). It is clear that specific angiotensin IV or angiotensin-(1–7) receptors do not mediate depressor effects in this model. The AT1 antagonist losartan (1 mg/kg) blocked both the pressor and depressor components of the angiotensin III response, whereas the AT2 antagonist PD 123319 (35 mg/kg) had no effect on either element of the response. The data obtained with the angiotensin receptor subtype-selective compounds, losartan and PD 123319, suggest that the depressor action is an AT1-mediated effect and give no indication that AT2 receptors could be involved. Paradoxically, the greater potency of angiotensin III as a vasodepressor belies the conclusion that the response is AT1-mediated, because AT1 receptors have a greater affinity for angiotensin II versus angiotensin III. (Hypertension. 2000;35:130-134.)

Key Words: angiotensin III receptors, angiotensin blood pressure rabbits losartan

Angiotensin II is well known as a potent vasoconstrictor, but many reports indicate that angiotensins exert vasodilator properties. Early in vitro work showed vasorelaxation by angiotensins in some vascular beds.1–3 and Campbell et al4 reported a vasodepressor phase after the characteristic pressor response to angiotensin II in conscious rabbits. We established that vasodilator prostaglandins contribute to the depressor response in conscious rabbits.5 Depressor or dilator responses to angiotensins are not duplicated by other vasopressors, tend to be manifested at high angiotensin concentrations, and might be prominent in specific vascular beds. For example, the cerebral and renal circulations appear susceptible to angiotensinogenic dilatation.2,6,7

New pharmacological tools became available in 19898,9 for the investigation of receptor mechanisms for depressor responses. Scheuer and Perrone10 reported depressor responses in barbiturate-anesthetized rats that were accentuated by the AT1 antagonist losartan and blocked by the putative AT2 antagonist PD 123319. Angiotensin III was a more potent depressor than was angiotensin II, and this is consistent with an AT1-mediated response because angiotensin III binds less effectively than angiotensin II at AT1 receptors but has a comparable affinity at AT2 receptors.11 An AT2-mediated dilator action is further supported by a study of Li et al,12 who reported that angiotensin III contractile responses in rat aorta were accentuated by PD 123177 but that angiotensin II and IV responses were unaffected. Other interesting differences between angiotensin II and III contractile responses in rat aorta were described by Sim and Yuan13: des-Asp angiotensin I attenuated the contractile responses to angiotensin III but potentiated the response to angiotensin II, and the authors state that the 2 peptides exert their effects at 2 different receptors. Vasodilatation by angiotensins in the cerebral circulation might occur by different mechanisms, in view of the fact that it appears to be blocked by both AT1 and AT2 antagonists and seems to be similar to arginine-induced vasodilatation.6,14

The various reports prompted us to reevaluate the angiotensin depressor response with the use of the conscious rabbit model described previously.5 Our experiments were designed to test the hypothesis that the depressor response is mediated by AT1 receptors and that depressor responses to angiotensin III will be more prominent than those produced by angiotensin II. Our experiments also address the hypothesis that angiotensinogenic depressor responses could be mediated by non-AT1/AT2 receptors, because of the experiments by Haberl6 described above and the observation that angiotensin IV and angiotensin-(1–7) have dilator or depressor activity.15,16

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Methods

Animal Preparation
Male New Zealand White rabbits (2.7 to 3.1 kg) were allowed food (Harlan Teklad 8630[W]) and water ad libitum and were monitored for steady weight gain before experimentation. All procedures were approved by the institutional committee governing animal care. A conscious rabbit was placed in a standard restraining box, and blood vessels in the ear were cannulated after application of the local anesthetic lidocaine (2% Xylocaine, Astra Pharmaceuticals). The central ear artery was cannulated with Clay-Adams PE-50 tubing (Beckton Dickinson & Co) after cutdown. The cannula contained heparinized saline (40 U/mL) and was connected to a Statham pressure transducer (model P23Db). Arterial blood pressure was monitored with a Grass polygraph (model 7D), and mean arterial pressure was obtained as the electronically damped pulse pressure. A PE-50 cannula was placed in the marginal ear vein via percutaneous puncture and was used for the administration of drugs. All drugs were infused in a volume of ≥0.1 mL and were flushed with 1 mL of saline.

Angiotensin peptides were given as 4 different doses in the initial experiments. Each dose was given at 20-minute intervals, and a 30-minute period was allowed between consecutive dose ranges. There is potential for a large dose of angiotensin to affect the response to a subsequent angiotensin bolus (tachyphylaxis). Randomization of dose sequence is one option that could be used to overcome sensitivity changes, but we elected not to use this approach because a large number of observations would be required for a valid analysis. A symmetric dose administration schedule was used instead; angiotensin peptides were administered in ascending and descending doses in alternate animals, and data obtained in this fashion were reproducible. Some experiments were conducted with a single repeating dose of angiotensin III (33 nmol/kg) in place of multiple dose sequences. These doses were given at 30-minute intervals.

Statistics
The data were evaluated as changes in mean arterial pressure and expressed as mean±SEM. The initial experiments were analyzed by ANOVA with repeated measures. In some experiments, responses before and after drug treatment were compared with identical protocols before and after vehicle administration. These data were processed by a 2-way ANOVA with repeated measures for the dose-sequence element (Abstat, Anderson-Bell Corp).

Materials
Angiotensin analogues were obtained from Bachem, and PD 123319 was from Research Biochemicals. Losartan was a gift from Merck & Co, Rahway, NJ.

Results

Comparison of Angiotensin Peptides
A biphasic response to angiotensin III is illustrated in Figure 1. There is animal-to-animal variation in the magnitude of the depressor response, and it was <8 mm Hg in a small number of animals; these were excluded from further study. Figure 2 details the blood pressure responses to a dose range of the heptapeptide. Three identical dose ranges were obtained at 90-minute intervals consecutively. Pressor-response tachyphylaxis can occur with large doses of angiotensin in the rabbit but is not evident here because the pressor responses to angiotensin III are well maintained for each consecutive dose-range administration. The depressor response to angiotensin III is less well maintained. There was a significant decline in the response to doses of 15 nmol/kg (separated by 90 minutes): 20±3, 11±2, and 11±3 mm Hg (P<0.05).

The depressor response to angiotensin III is considerably more prominent than the pressor response to angiotensin II, but the reverse is true for the pressor responses (Figure 1, bottom trace). Table 1 shows the blood pressure responses to the first dose range of angiotensin III depicted in Figure 2 and a dose range of angiotensin II at which the pressor potency is comparable to that of angiotensin III. Mean arterial blood pressure rises by 93±7 mm Hg after 5 nmol/kg angiotensin II, but the subsequent depressor phase (5±2 mm Hg) is small in relation to comparable doses of angiotensin III and is quite variable. Differences between angiotensin II and III are apparent also when the duration of the response is examined (Figure 1). The duration of the pressor response to 15 nmol/kg angiotensin III was 2.1±0.1 minutes (pressor response 82±4 mm Hg; see Table 1), whereas the duration of the response to angiotensin II at a comparable pressor dose (1.5 nmol/kg, pressor response 77±9 mm Hg) was significantly longer at 2.8±0.2 minutes (P<0.01, unpaired t test).
**AT₁ and AT₂ Subtype–Selective Antagonists**

The blood pressure responses to angiotensin III were evaluated before and after blockade of AT₁ receptors with losartan (Table 2). Losartan markedly attenuated the pressor response to angiotensin III, confirming blockade of AT₁ receptors. Surprisingly, the depressor responses were completely abolished also. The losartan data suggest that both pressor and depressor responses are mediated via AT₁ receptors, whereas the paradoxically higher potency of angiotensin III as a depressor suggests that the depressor response is mediated by a non-AT₁ receptor.

The paradox was evaluated further by examining the effect of PD 123319, a selective AT₂ antagonist, on the depressor response. This experiment was conducted differently: 3 single large doses of angiotensin III (33 nmol/kg) were given 30 minutes apart, and PD 123319 (35 mg/kg, n=8) was given 2 minutes before the second dose (Figure 3). A parallel protocol in which vehicle was substituted for PD 123319 is shown also (n=8). Pressor responses to angiotensin III were unaffected in any treatment group. A 2-way ANOVA with repeated measures revealed a significant decline in the depressor response with time (P=0.008), but there was no significant difference between the PD 123319 group and the control group. There is no effective method to test AT₂ blockade, but the amount of PD 123319 given (35 mg/kg) was larger than that used by many investigators. Angiotensin III responses were tested at 2 and 30 minutes after PD 123319 administration to maximize the likelihood of AT₂ receptor blockade. The number of observations in the PD 123319 group (n=4) was limited by the extreme cost of the compound.

**Discussion**

The results confirm an angiotensin-induced depressor response in the conscious rabbit model as described previously. The response is evident at high concentrations of angiotensin (>0.5 nmol/kg), even in milligram quantities.

**TABLE 1. Pressor and Depressor Responses to Angiotensin Analogues**

<table>
<thead>
<tr>
<th>Angiotensin III (n=6)</th>
<th>Dose, nmol/kg</th>
<th>Pressor, mm Hg</th>
<th>Depressor, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>28±2</td>
<td>32±2</td>
<td>3±2</td>
</tr>
<tr>
<td>1.5</td>
<td>43±2</td>
<td>56±5</td>
<td>7±3</td>
</tr>
<tr>
<td>5.0</td>
<td>64±3</td>
<td>77±9</td>
<td>5±2</td>
</tr>
<tr>
<td>15</td>
<td>82±4</td>
<td>93±7</td>
<td>5±2</td>
</tr>
</tbody>
</table>

**TABLE 2. Effect of Losartan on Pressor and Depressor Responses to Angiotensin III**

<table>
<thead>
<tr>
<th>Angiotensin III Responses, mm Hg</th>
<th>1 nmol/kg</th>
<th>3 nmol/kg</th>
<th>7 nmol/kg</th>
<th>20 nmol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pressor</td>
<td>30±2</td>
<td>51±4</td>
<td>72±4</td>
<td>87±3</td>
</tr>
<tr>
<td>Depressor</td>
<td>0</td>
<td>4±2</td>
<td>12±4</td>
<td>22±6</td>
</tr>
<tr>
<td><strong>Phase 2 (after losartan)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pressor</td>
<td>17±3</td>
<td>24±3</td>
<td>32±3</td>
<td>48±2</td>
</tr>
<tr>
<td>Depressor</td>
<td>0</td>
<td>0</td>
<td>3±2</td>
<td></td>
</tr>
<tr>
<td><strong>Phase 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pressor</td>
<td>22±2</td>
<td>29±3</td>
<td>34±3</td>
<td>53±3</td>
</tr>
<tr>
<td>Depressor</td>
<td>0</td>
<td>0</td>
<td>2±2</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=6).

Doses of angiotensin III were administered at 20-minute intervals, and each of the 3 phases of the experiment were 90 minutes in duration. Thirty minutes was allowed between the last dose of angiotensin III in one phase and the first dose in the next phase of the experiment. Losartan (1 mg/kg) was given at the midpoint of the 30-minute period between the first and second phases.
Scheuer and Perrone\textsuperscript{10} that angiotensin III is a more potent depressor than is angiotensin II. The depressor phase follows the well-known pressor response after an intravenous bolus of the peptide. The depressor phase was reproducible with repeated administration of angiotensin III at the smaller doses, but the magnitude of the response to the larger dose (15 nmol/kg) fell significantly from an initial value of 20±3 mm Hg to 11±2 and 11±3 mm Hg. The accompanying pressor components were well maintained, which suggests that the mechanisms for the pressor and depressor responses are different. A qualitative inspection of the responses in Figure 1 indicates that a large depressor component of the angiotensin III response is associated with a truncated pressor phase. It could be argued that in these circumstances an abbreviated pressor phase accentuates the expression of a depressor component, and this notion might be supported if angiotensin III had a shorter half-life than angiotensin II. There is no information on the biological half-lives of the peptides in the rabbit, but they are comparable in the rat (14 versus 16 seconds; angiotensin III versus II, respectively).\textsuperscript{20} Moreover, when the depressor phase is blocked by cyclooxygenase inhibitors, the pressor component is prolonged.\textsuperscript{2} It seems likely then that the depressor component has a slow onset and a longer duration but can dominate and mask the late pressor phase. Studies with low-dose infusions of angiotensin II indicate that an elevation in mean arterial pressure of only 8 mm Hg incorporates a depressor element of 3 to 6 mm Hg, which can be revealed by prostaglandin synthesis inhibition.\textsuperscript{23} In humans, angiotensin pressor responses of only 20 mm Hg generate a significant depressor component, which intensifies during pregnancy.\textsuperscript{22} This provides evidence that physiological concentrations of angiotensin could exert meaningful depressor activity, although it is not evident with low-dose bolus administration.

The desensitization of the depressor action relative to the pressor response and the higher depressor potency of angiotensin III provide compelling evidence that the 2 responses are mediated by different receptor mechanisms. The hypothesis is further consolidated by the findings of Scheuer and Perrone\textsuperscript{10} showing that the depressor phase in rats is mediated by AT\textsubscript{1} receptors and the studies of Endo et al\textsuperscript{7} implicating AT\textsubscript{2} receptors in renal vasodilatation. Additionally, the AT\textsubscript{2}-selective compound PD 123319 enhances the contractile response to angiotensin III, but not angiotensin II, in rat aorta.\textsuperscript{12} and des-Asp\textsuperscript{1} angiotensin I attenuates the contractile response to angiotensin III, but not angiotensin II, in the same tissue.\textsuperscript{13} In view of this overwhelming evidence, it was astonishing that in our experiments losartan abolished the depressor component of the angiotensin III response, whereas PD 123319 had no effect. The dose of losartan was less than that used by most investigators; it is highly improbable that this dose influenced AT\textsubscript{2} receptors because residual pressor responses indicate incomplete blockade even at AT\textsubscript{1} receptors. It is difficult to evaluate the in vivo effect of PD 123319 on AT\textsubscript{2} receptors in the absence of a good biological response to test antagonism. Moreover, PD 123319 has a short biological half-life (22 minutes; J. Keiser, personal communication, 1998). These problems were addressed by giving PD 123319 at 3.5 times the amount given in the experiments reported by Scheuer and Perrone, and the responses to a single dose of angiotensin III were examined 2 to 30 minutes after PD 123319 administration to maximize the window for blockade of AT\textsubscript{2} receptors.

The argument that the depressor response is losartan sensitive but mechanistically distinct from AT\textsubscript{1} actions might be resolved in several ways. One possibility is that the depressor response could be mediated by a non-AT\textsubscript{1}/AT\textsubscript{2} receptor that happens to be losartan sensitive and PD 123319 insensitive. The most likely candidates would be receptors that use angiotensin-(1–7) or angiotensin IV as primary agonists. Biological actions of angiotensin-(1–7) might be mediated by unique non-AT\textsubscript{1}/AT\textsubscript{2} receptors,\textsuperscript{18} and a depressor response for angiotensin-(1–7) has been reported.\textsuperscript{16,23} Angiotensin IV also functions via non-AT\textsubscript{1}/AT\textsubscript{2} receptors\textsuperscript{19} and can cause renal vasodilatation.\textsuperscript{15} Angiotensin IV was 30 to 50 times less potent than angiotensin III as a pressor agent and was equally less potent as a depressor in our hands. This argues against a depressor action mediated via receptors with a high affinity for angiotensin IV and actually strengthens the case for a depressor action linked to AT\textsubscript{1} receptors. Angiotensin-(1–7) produced weak pressor responses even in milligram quantities, and there was no evidence of a depressor response. Thus, the depressor response in the rabbit model is not mediated by receptors with affinity for angiotensin-(1–7).

It is difficult to define the mechanism of the depressor response reported in the present study. It is not a reflex phenomenon because it does not occur with other pressors and is relatively less prominent with angiotensin II, despite its superlative pressor action. We discounted depressor actions mediated by the putative non-AT\textsubscript{1}/AT\textsubscript{2} receptors responsible for responses to angiotensin-(1–7) and angiotensin IV, but we cannot exclude the possibility of further angiotensin receptors that are, as yet, uncharacterized. Despite evidence to the contrary, we cannot positively exclude an AT\textsubscript{1}-mediated depressor response. For example, acute elevations in blood pressure might facilitate the transfer of angiotensin peptides across the blood-brain barrier, where angiotensin III could initiate AT\textsubscript{2}-mediated vasodilatation. If PD 123319 does not adequately access central nervous system tissue, the AT\textsubscript{2}-mediated depressor action would be unresponsive to circulating PD 123319 but blocked by the AT\textsubscript{1} antagonist by virtue of its antihypertensive properties. Another tenuous explanation is that the depressor response is mediated by AT\textsubscript{2} receptors but that the response does not exhibit a classic structure-activity profile. This could occur if internalized AT\textsubscript{1}/angiotensin III initiates atypical postreceptor events or angiotensin III activates unidentified receptors that synergize with AT\textsubscript{1}.

When the findings of depressor/dilator actions of angiotensins reported by various laboratories are taken into consideration, the evidence supports and refutes roles for prostaglandins, nitric oxide, AT\textsubscript{1}, AT\textsubscript{2}, and non-AT\textsubscript{1}/AT\textsubscript{2} receptors. It appears likely that multiple mechanisms are operative in various species and various locations.

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References


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