[Pro^{11}, D-Ala^{12}]angiotensin I has rapid onset vasoconstrictor activity in the cat

ETOI A. GARRISON, HUNTER C. CHAMPION, AND PHILIP J. KADOWITZ
Department of Pharmacology, Tulane University School of Medicine, New Orleans, Louisiana 70112

Garrison, Etoi A., Hunter C. Champion, and Philip J. Kadowitz. [Pro^{11}, D-Ala^{12}]angiotensin I has rapid onset vasoconstrictor activity in the cat. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E1059–E1064, 1997.—Responses to the synthetic substrate [Pro^{11}, D-Ala^{12}]angiotensin I were investigated in the hindlimb vascular bed of the cat, a system in which local angiotensin-converting enzyme activity is high. Under constant-flow conditions, injections of [Pro^{11}, D-Ala^{12}]angiotensin I into the perfusion circuit in doses of 1–300 µg caused dose-related increases in perfusion pressure that were rapid in onset and that were not changed by the presence of a time-delay coil in the perfusion circuit upstream from the site of peptide injection. The synthetic substrate was 100-fold less potent than angiotensin I and II, and responses to [Pro^{11}, D-Ala^{12}]angiotensin I were not altered by captopril in a dose that inhibited pressor responses to angiotensin I but did not alter responses to angiotensin II. Responses to [Pro^{11}, D-Ala^{12}]angiotensin I, angiotensin I, and angiotensin II were inhibited by DUP-532 and candesartan but were not altered by the angiotensin AT_{2} receptor antagonist PD-123319. The present data show that [Pro^{11}, D-Ala^{12}]angiotensin I has significant vasoconstrictor activity in the hindlimb vascular bed of the cat and suggest that responses are mediated by the activation of AT_{1} receptors and that activation of AT_{2} receptors is not involved. The present data show that the onset of responses to [Pro^{11}, D-Ala^{12}]angiotensin I and angiotensin II are similar and are not dependent on the action of the angiotensin-converting enzyme. The present data suggest that conversion of the synthetic substrate to an active peptide occurs rapidly within the hindlimb vascular bed or that the peptide may have direct AT_{1} receptor-stimulating activity.

hindquarters vascular bed; angiotensin peptides; vasoconstrictor responses; angiotensin-converting enzyme; AT_{1} receptors; DUP-532; PD-123319

THE ROLE of the renin-angiotensin system (RAS) in the regulation of blood pressure, sodium, and water homeostasis is well established (6, 22, 23). In recent years, components of the RAS have been identified in several target organs, including the heart, brain, adrenal gland, kidney, and the vasculature (2, 5, 26). The identification of a tissue RAS suggests that regional angiotensin-converting enzyme (ACE) and locally formed angiotensin peptides may play a role in the regulation of vascular tone. Although it is historically believed that ACE is the primary enzyme responsible for the conversion of the biologically inactive angiotensin I to the active peptide angiotensin II, several studies have demonstrated the existence of ACE-independent angiotensin II-forming pathways (14, 18, 28).

In the human heart, ~80% of the angiotensin II-forming activity has been attributed to a chymostatin-sensitive serine proteinase (chymase), whereas ACE-dependent angiotensin II formation has been reported to account for ~20% of the generation of the active peptide (14, 29, 28). Although ACE has been reported to be the primary enzyme responsible for the formation of angiotensin II in isolated arteries of the rat (21) and rabbit (20), it was found to be less important in isolated arteries of the dog (18) and humans (20). In isolated human gastroepiploic and mesenteric arteries, only 30–40% of angiotensin II formation has been attributed to ACE, whereas ~60–70% of angiotensin II formation was attributed to the chymase pathway (19). These results suggest that there may be species differences with regard to the role of non-ACE pathways in the formation of angiotensin II in the regional vascular bed.

The chymase enzyme has also been called human heart chymase, angiotensin I convertase (16, 30), and chymostatin-sensitive angiotensin II-generating enzyme (14, 20). Unlike ACE, chymase does not degrade bradykinin or substance P and is reported to be more specific than ACE for the conversion of angiotensin I to angiotensin II with a specificity constant of 198 min^{-1} (13, 16, 30). Chymase activity is reported to be inhibited by the serine protease inhibitors, soybean trypsin inhibitor, chymostatin, and phenylmethylsulfonfluoride (30). Chymase, unlike the angiotensin II-generating enzymes, tonin, cathepsin G, and trypsin, does not degrade angiotensin II or produce angiotensin II directly from angiotensinogen (11, 13, 16, 30). In addition, chymase immunoreactivity and mRNA have been identified in cardiac endothelial cells, fibroblasts, and mast cells (27).

[Pro^{11}, D-Ala^{12}]angiotensin I is a novel angiotensin I analog that has been reported to be an ACE-resistant substrate for chymase (11–13, 16). This angiotensin I analog contains a penultimate proline, which has been reported to prevent carboxy terminal cleavage of the peptide by ACE, and carboxy terminal d-alanine, which has been reported to prevent carboxy terminal degradation of the peptide by carboxy peptidases (11–13). In the isolated dog superior mesenteric artery, the [Pro^{11}, D-Ala^{12}]angiotensin I-induced contraction was not altered by captopril and was significantly attenuated in the presence of the serine protease inhibitor, chymostatin (16). In marmosets, systemic pressor responses to [Pro^{11}, D-Ala^{12}]angiotensin I were either slightly attenuated or not altered by captopril (16). In conscious baboons, pressor responses to [Pro^{11}, D-Ala^{12}]angiotensin I were significantly reduced by angiotensin AT_{1} receptor antagonists. [Pro^{11}, D-Ala^{12}]angiotensin I is reported to have low affinity for the angiotensin II receptor [50% inhibitory concentration (IC_{50}) > 10 µM] (13), suggesting that pressor responses to the ACE-resistant substrate may be due to its conversion by chymase to angiotensin II. Little, if anything, however, is known about responses to local intra-arterial injections of
[Pro$^{11}$,D-Ala$^{12}$]angiotensin I in the regional vascular bed of the cat. The present study was, therefore, undertaken to investigate responses to [Pro$^{11}$,D-Ala$^{12}$]angiotensin I and to determine the role of ACE and angiotensin AT$_1$ and AT$_2$ receptors in mediating or modulating responses to the synthetic peptide in the hindlimb circulation of the cat.

METHODS

Fifty-one adult cats (2.5–4.0 kg) unselected as to sex were sedated with ketamine hydrochloride (10–20 mg/kg im) and anesthetized with pentobarbital sodium (30 mg/kg iv). Additional doses of pentobarbital were given as needed to maintain a uniform level of anesthesia. The trachea was cannulated, and animals were ventilated with a Harvard model 607 ventilator at a volume of 40–60 ml at 15–22 breaths/min, so that arterial blood gases and pH measured with a Corning 178 analyzer were in the physiological range. Catheters were placed into an external jugular vein for the intravenous administration of drugs and into the carotid artery for the measurement of systemic arterial pressure. For constant-flow perfusion of the hindlimb vascular bed, a 3- to 4-cm segment of the abdominal aorta was approached through a lateral midline incision and cleared of surrounding connective tissue. Branches of the aorta distal to the origin of the external iliac arteries were ligated to restrict blood flow to the hindlimb. After administration of heparin sodium (1,500 U/kg iv), the aorta was ligated and catheters were inserted proximal and distal to the ligature. Blood was withdrawn from the proximal catheter and pumped at a constant rate by a Sigmamotor model T-8 pump into the distal aortic catheter. Hindquarters perfusion pressure and systemic arterial pressure were measured with Statham P23 pressure transducers and recorded on a Grass model 7 polygraph. The flow rate was set so that hindlimb perfusion pressure approximated systemic arterial pressure and was not changed throughout the course of the experiment. The flow rate averaged 28 ± 4 ml/min. The hindlimb vascular bed was denervated by ligating and cutting the lumbar sympathetic chain ganglia between L$_3$ and L$_4$. The pump-off occlusion pressure approached small vein pressure, indicating that collateral inflow to the hindlimb vascular bed was minimal. These procedures have been described previously (8) and have been approved by the Tulane University Animal Use Committee.

In the present study, four sets of experiments were carried out. In the first set of experiments, responses to [Pro$^{11}$,D-Ala$^{12}$]angiotensin I, angiotensin I and II, and norepinephrine were compared when doses were expressed on a nanomole basis. In the range of doses used for each peptide, responses were reproducible, and tachyphylaxis was not observed. To determine if activation or conversion to an active peptide occurs at a distal site, such as in the lung, and if recirculation of an active angiotensin peptide contributes to the increase in hindlimb perfusion pressure in response to intra-arterial injection of [Pro$^{11}$,D-Ala$^{12}$]angiotensin I, the time course of hindlimb pressor response to [Pro$^{11}$,D-Ala$^{12}$]angiotensin I was compared in experiments in which a standard perfusion circuit with a 5- to 10-s delay or appearance time was used. The agonists used in these studies were administered as bolus injections directly into the hindlimb perfusion circuit distal to the pump in a random sequence in small volumes (30–100 µl). Rapid bolus injection of the saline vehicle for the peptides had no significant effect on hindlimb perfusion pressure. The antagonists used in these studies were injected intravenously, and responses to the angiotensin peptides were compared before and beginning 10–20 min after administration of the receptor antagonists or ACE inhibitor. Angiotensin I and II, norepinephrine bitartrate (Sigma Chemical, St. Louis, MO), angiotensin IV (Bachem, Torrance, CA), and [Pro$^{11}$,D-Ala$^{12}$]angiotensin I (generously provided by Dr. Leonard Loose of Pfizer, Groton, CT) were dissolved in 0.9% NaCl. Candesartan (CV-11974 Astra Hassle, Molndal, Sweden) was dissolved in a 2 N Na$_2$CO$_3$–0.9% NaCl solution (1:20). The hemodynamic data are expressed as means ± SE and were analyzed using a one-way analysis of variance and Scheffe’s F-test or paired t-test (25). A P value of <0.05 was used as the criterion for statistical significance.

RESULTS

Under constant-flow conditions, injections of [Pro$^{11}$,D-Ala$^{12}$]angiotensin I in the hindlimb perfusion circuit caused dose-related increases in hindlimb perfusion pressure (Fig. 1). [Pro$^{11}$,D-Ala$^{12}$]angiotensin I was ~100-fold less potent than angiotensin I and II and was similar in potency to norepinephrine in increasing perfusion pressure in the hindlimb vascular bed of the cat when doses are expressed on a nanomole basis to
take molecular weight into account. The time course of the increase in hindlimb perfusion pressure in response to [Pro\(^{11}\),D-Ala\(^{12}\)]angiotensin I and the effects of a time-delay coil positioned upstream from the site of injection of the peptide in the perfusion circuit on the hindlimb vasoconstrictor response to the angiotensin I analog is shown in Fig. 2. The time course of the increase in perfusion pressure in response to [Pro\(^{11}\),D-Ala\(^{12}\)]angiotensin I in a dose of 30 µg and angiotensin II in a dose of 0.3 µg was similar in that pressor responses to both peptides reached a peak in 20–40 s and perfusion pressure returned to baseline value within a 370- to 400-s period (Fig. 2). The addition of a timedelay coil to the inlet side of the perfusion circuit upstream from the site of injection had no significant effect on the time course or magnitude of the pressor response to [Pro\(^{11}\),D-Ala\(^{12}\)]angiotensin I in the hindlimb vascular bed (Fig. 2).

To investigate the role of ACE in mediating the pressor response to [Pro\(^{11}\),D-Ala\(^{12}\)]angiotensin I, the effects of captopril were assessed, and these data are summarized in Fig. 3. After the administration of the ACE inhibitor in an intravenous dose of 4 mg/kg, the increases in hindlimb perfusion pressure in response to [Pro\(^{11}\),D-Ala\(^{12}\)]angiotensin I and angiotensin II were not changed, whereas the pressor response to angiotensin I was significantly reduced (Fig. 3). Treatment with captopril in an intravenous dose of 4 mg/kg decreased baseline hindlimb perfusion pressure and systemic arterial pressure (Table 1).

The role of the angiotensin \(\text{AT}_1\) receptor in mediating the pressor response to [Pro\(^{11}\),D-Ala\(^{12}\)]angiotensin I was investigated, and these results are summarized in Fig. 4. After administration of DUP-532 in an intravenous dose of 100 µg/kg or candesartan in an intravenous dose of 300 µg/kg, pressor responses to [Pro\(^{11}\),D-Ala\(^{12}\)]angiotensin I and angiotensin II were significantly reduced, whereas pressor responses to norepinephrine were unaltered (Fig. 4). Treatment with DUP-532 (100 µg/kg iv) and candesartan (300 µg/kg iv) had no significant effect on baseline hindlimb or systemic arterial pressure (Table 1).

Role of the angiotensin \(\text{AT}_2\) receptor. The role of angiotensin \(\text{AT}_2\) receptors in mediating or modulating pressor responses to [Pro\(^{11}\),D-Ala\(^{12}\)]angiotensin I was investigated in the hindlimb vascular bed of the cat, and the results of this series of experiments are summarized in Table 1.

### Table 1. Changes in baseline mean vascular pressures in the cat

<table>
<thead>
<tr>
<th></th>
<th>Hindlimb Perfusion Pressure, mmHg</th>
<th>Systemic Arterial Pressure, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>127 ± 10</td>
<td>116 ± 8</td>
</tr>
<tr>
<td>Captopril (4 mg/kg iv)</td>
<td>115 ± 11*</td>
<td>96 ± 7*</td>
</tr>
<tr>
<td>Control</td>
<td>120 ± 8</td>
<td>124 ± 7</td>
</tr>
<tr>
<td>DUP-532 (100 µg/kg iv)</td>
<td>126 ± 7</td>
<td>122 ± 8</td>
</tr>
<tr>
<td>Control</td>
<td>123 ± 10</td>
<td>120 ± 9</td>
</tr>
<tr>
<td>Candesartan (300 µg/kg iv)</td>
<td>118 ± 9</td>
<td>116 ± 8</td>
</tr>
<tr>
<td>Control</td>
<td>119 ± 9</td>
<td>114 ± 7</td>
</tr>
<tr>
<td>PD 123319 (5 mg/kg iv)</td>
<td>116 ± 9</td>
<td>114 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of experiments. *P < 0.05.
rized in Fig. 5. After administration of the AT<sub>2</sub> receptor antagonist PD-123319 in an intravenous dose of 5 mg/kg, pressor responses to [Pro<sup>11</sup>, D-Ala<sup>12</sup>]angiotensin I, angiotensin II, or norepinephrine were not altered (Fig. 5). PD-123319 did not alter systemic arterial or hindlimb perfusion pressure (Table 1).

**DISCUSSION**

The present results show that the angiotensin I analog [Pro<sup>11</sup>,D-Ala<sup>12</sup>]angiotensin I produced dose-related increases in perfusion pressure in the hindlimb vascular bed of the cat. Inasmuch as blood flow to the hindlimb was maintained constant, the increases in perfusion pressure in response to the ACE-resistant angiotensin I analog reflect increases in hindlimb vascular resistance. When doses are expressed on a nanomole basis to take molecular weight into account, [Pro<sup>11</sup>, D-Ala<sup>12</sup>]angiotensin I was ~100-fold less potent than angiotensin II in increasing hindlimb perfusion pressure in the cat. The site at which [Pro<sup>11</sup>, D-Ala<sup>12</sup>]angiotensin I is converted into an active peptide was investigated in experiments in which a time-delay coil was added to the inlet side of the hindlimb perfusion circuit upstream from the site of injection. The results of these experiments show that the presence of the time-delay coil had no significant effect on the time course of the increase in perfusion pressure in response to [Pro<sup>11</sup>, D-Ala<sup>12</sup>]angiotensin I, suggesting that the substrate may be converted into an active peptide within the hindlimb vascular bed. The time course of the increase in perfusion pressure in response to [Pro<sup>11</sup>, D-Ala<sup>12</sup>]angiotensin I and angiotensin II was rapid and similar. These data suggest that [Pro<sup>11</sup>, D-Ala<sup>12</sup>]angiotensin I may be rapidly converted to an active peptide at or near the site of action, which has been observed for angiotensin II in the rat cremaster muscle to be small arterioles upstream from the capillary bed (7). The rapid time course of the increase in pressure in response to the ACE-resistant analog may also be interpreted to suggest that [Pro<sup>11</sup>, D-Ala<sup>12</sup>]angiotensin I itself may directly stimulate the AT<sub>1</sub> receptor.

Increases in hindlimb perfusion pressure in response to [Pro<sup>11</sup>, D-Ala<sup>12</sup>]angiotensin I and angiotensin II were not altered by administration of captopril in a dose that significantly reduced the pressor response to angiotensin I, suggesting that ACE does not play a significant role in mediating the hindlimb pressor response to [Pro<sup>11</sup>, D-Ala<sup>12</sup>]angiotensin I. The observation that responses to angiotensin I are markedly inhibited by captopril suggests that ACE is the major pathway for converting angiotensin I into an active peptide within the hindlimb vascular bed. These results confirm studies in the marmoset in which pressor responses to [Pro<sup>11</sup>, D-Ala<sup>12</sup>]angiotensin I were either slightly attenuated or not altered by captopril (16), and the results of biochemical studies in which [Pro<sup>11</sup>, D-Ala<sup>12</sup>]angiotensin I was found to be resistant to ACE hydrolysis and was converted to angiotensin II by chymase (10). The observation that captopril decreased systemic arterial and hindquarters perfusion pressure, whereas candesartan and losartan had little if any effect, may suggest that...
the hypotensive effect of the ACE inhibitor in the cat is due mainly to enhanced formation of bradykinin.

Nonpeptide AT<sub>1</sub> receptor antagonists were used to investigate the angiotensin II receptor subtype mediating pressor responses to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I. Pressor responses to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I were significantly reduced by DUP-532 and candesartan in doses that attenuated pressor responses to angiotensin II but that had no effect on pressor responses to norepinephrine in the hindlimb vascular bed. The results showing that responses to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I are attenuated by DUP-532 and candesartan suggest that vasoconstrictor responses to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I, like responses to angiotensin II and angiotensin IV [angiotensin II-3–8], are mediated by the activation of AT<sub>1</sub> receptors on resistance vessel elements in the hindlimb vascular bed of the cat.

The putative angiotensin AT<sub>2</sub> receptor antagonist PD-123319 was used to study the role of angiotensin AT<sub>2</sub> receptors in mediating or modulating responses to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I in the hindlimb vascular bed of the cat (3). It has been reported that angiotensin II and angiotensin III (des-Asp<sup>1</sup>-angiotensin II) produce biphasic changes in systemic arterial pressure in the anesthetized rat and that, during AT<sub>1</sub> receptor blockade, increases in pressure are eliminated, whereas depressor responses were enhanced. Moreover, it has also been shown in the rat that AT<sub>1</sub> receptor blockade eliminates responses to the angiotensin peptides, whereas AT<sub>2</sub> receptor blockade enhanced pressor responses (24). In the present experiments, biphasic changes in hindlimb perfusion pressure in response to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I or angiotensin II were not observed. PD-123319 in an intravenous dose of 5 mg/kg did not alter pressor responses to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I, angiotensin II, or norepinephrine. Although a positive control agonist for the AT<sub>2</sub> receptor is not available, these data may be interpreted to suggest that angiotensin AT<sub>2</sub> receptors do not mediate a depressor response or modulate pressor responses to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I and are consistent with previous studies in the hindlimb vascular bed of the cat (8). The reason for the difference in results in the present study and previous studies in the literature is uncertain but may involve differences in species, vascular bed studied, or experimental procedures employed.

In the present study, pressor responses to angiotensin I, when administered in an intra-arterial dose of 0.3 μg, were significantly reduced (~80%), but not abolished, by captopril (4 mg/kg iv). We have previously shown that the administration of a second 4 mg/kg dose of captopril did not produce an additional inhibitory effect in the hindlimb pressor response to angiotensin I (8). These results may be interpreted to suggest that ACE is the major pathway and that non-ACE pathways, such as angiotensin I-converting enzyme, may play a lesser role in the conversion of angiotensin I to angiotensin II within the hindlimb vascular bed of the cat. It is also possible, however, that a minor component of the angiotensin I-induced increase in hindlimb perfusion pressure may be due to direct AT<sub>1</sub> receptor activation by angiotensin I. Aiken and Vane (1) reported that ~1–4% of the angiotensin I activity persisted after ACE inhibition and were among the first to suggest that angiotensin I may have direct biological activity. Several recent studies have shown, however, that, depending on the species, pressor responses to angiotensin I can be abolished by an ACE inhibitor alone or by the administration of an ACE inhibitor and chymostatin, a serine proteinase inhibitor (14, 16, 18, 19). These results suggest that a direct action by angiotensin I on vascular smooth muscle is unlikely, and it is also possible that residual tissue ACE activity sufficient to produce a pressor response to angiotensin I may remain in patients treated with ACE inhibitors. Several studies have shown that, although ACE inhibitors initially reduce plasma ACE activity, during chronic treatment, plasma angiotensin II levels increase over time (11, 12, 17). These observations may suggest the presence of an alternate pathway for the formation of angiotensin II.

The present study is the first to demonstrate ACE-resistant, angiotensin AT<sub>1</sub>-receptor-mediated vasoconstrictor responses to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I in the regional vascular bed of the cat. [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I has been reported in biochemical studies to be a substrate for chymase with little to no direct activation of the angiotensin AT<sub>1</sub> receptor (IC<sub>50</sub> > 10 μM) (13). The present results may be interpreted to suggest that a non-ACE pathway may play a role in mediating the responses to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I in the hindlimb vascular bed of the cat and are consistent with studies in the hamster heart (9), hamster cheek pouch (4), and the isolated pulmonary and mesenteric vessels of the monkey and dog (19). However, the possibility that [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I has direct AT<sub>1</sub> receptor-stimulating activity cannot be ruled out by the results of time course experiments in the present study. Moreover, the rapid onset of the pressor response to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I may suggest that conversion to angiotensin II is very rapid, since the time course of responses to angiotensin I, angiotensin II, and [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I are very similar, or that the peptide may itself possess direct AT<sub>1</sub> receptor-stimulating activity.

It has been suggested that angiotensin I is converted to angiotensin II by ACE in the luminal compartment, whereas [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I undergoes conversion to angiotensin II by chymase in an extraluminal compartment (4). The results of the present study showing that [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I is less potent than angiotensin II may be interpreted to suggest that access of [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I to the extraluminal compartment is limited. However, data from the present study show that the response to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I is rapid in onset and similar in time course to the response to angiotensin I and angiotensin II. If there were a delay in the conversion of [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I to angiotensin II due to slow diffusion to the extraluminal compartment, a delay in the onset of the response to [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I would be expected when compared with the time course of the response to angiotensin I. The reason for these differences is uncertain, but may suggest that [Pro<sup>11</sup>,D-Ala<sup>12</sup>]-angiotensin I has direct AT<sub>1</sub> receptor-mediated
vasoconstrictor activity. A more definitive conclusion regarding the role of the chymase enzyme in mediating responses to [Pro^{11},D-Ala^{12}]angiotensin I within the regional vascular bed of the cat must await the development of a specific chymase inhibitor that can be used in vivo experiments (12, 16).

In summary, the results of the present study show that [Pro^{11},D-Ala^{12}]angiotensin I has significant vasoconstrictor activity in the hindlimb vascular bed of the cat. The time course of the responses to [Pro^{11},D-Ala^{12}]angiotensin I, when compared with the response to angiotensin II and the absence of an effect of the time-delay coil on the hindlimb pressor response, suggests that the substrate may be rapidly and efficiently converted to an active peptide within the hindlimb vascular bed of the cat. This cannot be ruled out at the present time.

The authors gratefully acknowledge Dr. Leland Loose of Pfizer for providing [Pro^{11},D-Ala^{12}]angiotensin I and Janice Ignarro for editorial assistance. E. Garrison is supported by a Minority Predoctoral Fellowship Grant from the National Institutes of Health. This work was supported in part by National Heart, Lung, and Blood Institute Grant HL-15580 and a grant from the American Heart Association of Louisiana.

Address for reprint requests: P. J. Kadowitz, Dep. of Pharmacology, Tulane Univ. School of Medicine, 1430 Tulane Ave., New Orleans, LA 70112.

Received 24 April 1997; accepted in final form 7 August 1997.

REFERENCES


