Responses to angiotensin peptides are mediated by AT$_1$ receptors in the rat

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Champion, Hunter C., Marc A. Czapla, and Philip J. Kadowitz. Responses to angiotensin peptides are mediated by AT$_1$ receptors in the rat. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E115–E123, 1998.—The effects of the functional AT$_1$ and AT$_2$ receptor antagonists candesartan and PD-123,319 on hemodynamic responses to angiotensin peptides were investigated in the anesthetized rat. Injections of angiotensin II and III caused dose-related increases in systemic arterial and in hindquarters perfusion pressure that were reduced in an insurmountable manner by candesartan. Pressor responses to angiotensin IV were also attenuated, and a vasodepressor or vasodilator response to the angiotensin peptides was not unmasked by the AT$_1$ receptor antagonists candesartan or losartan. The AT$_2$ receptor antagonist PD-123,319 had no significant effect on increases in systemic arterial and hindquarters perfusion pressure in response to the angiotensin peptides. Pressor responses to angiotensin peptides were not altered by adrenergic nerve terminal and $\alpha$-receptor blocking agents or by the cyclooxygenase inhibitor sodium medofenamate but were increased by an inhibitor of nitric oxide synthase. The present results suggest that pressor responses to the angiotensin peptides are mediated by the activation of AT$_1$ receptors and that AT$_2$ receptors, the adrenergic system, or cyclooxygenase products do not appear to modulate hemodynamic responses to the angiotensin peptides in the anesthetized rat.

arterial pressure; candesartan; PD-123,319; angiotensin II, III, IV; vasoconstrictor-vasodilator responses; hindquarters vascular bed; adrenergic system

ANGIOTENSIN II is a potent vasoactive peptide formed from angiotensin I that plays an important role in the regulation of vasomotor tone, sodium, and water homeostasis (13, 16–18, 21, 22, 27). Vasoconstrictor responses to angiotensin II are mediated by the activation of angiotensin AT$_1$ receptors in a number of vascular beds in a variety of species (1, 5, 6, 10, 12, 13, 18, 25, 30). Angiotensin II can be converted to angiotensin III and IV by aminopeptidases, and angiotensin II and III have similar AT$_1$ receptor-mediated pressor activity in the regional vascular bed of the cat (5, 9, 15, 16, 19). Angiotensin IV appears to have full agonist activity in the regional vascular bed of the cat but has 100-fold less affinity for the AT$_1$ receptor (5, 8, 9, 13, 15, 16). Although angiotensin II and III bind to both AT$_1$ and AT$_2$ receptor subtypes, it appears that most physiological responses are mediated by AT$_1$ receptor activation and little, if anything, is known about the functional significance of AT$_2$ receptors in the cardiovascular system (1, 2, 5–7, 11, 13, 20, 24, 25). It has been reported that angiotensin II can produce biphasic changes in systemic arterial pressure in the rabbit and rat (4, 14, 23, 24, 28). It has also been reported that AT$_2$ receptors mediate the depressor phase of a biphasic response to angiotensin II and III in the anesthetized rat (24). In contrast, it has been reported that the AT$_2$ receptor antagonist PD-123,319 in intravenous doses up to 20 mg/kg did not modify vasoconstrictor responses to angiotensin peptides in the regional vascular bed of the cat (5). The difference in results in regard to the biphasic character of the response to angiotensin II and III and the effects of the AT$_2$ receptor antagonist on hemodynamic responses to the angiotensin peptides may be due to differences in species (24). The present study was, therefore, undertaken to investigate responses to angiotensin II, III, and IV and the effects of the AT$_1$ and AT$_2$ receptor antagonists on hemodynamic responses to angiotensin peptides in the systemic vascular bed of the rat.

MATERIALS AND METHODS

Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) of either sex, weighing 305–490 g, were used in these experiments and were housed in groups in a room maintained at 70°F with a 12:12-h light-dark cycle. The rats were fed Laboratory Rodent Diet no. 5001 (P.M.I Feeds, St. Louis, MO), and water was available ad libitum. The animals were anesthetized with pentobarbital sodium (Nembutal sodium solution, 50 mg/kg ip; Abbott Laboratories, North Chicago, IL) or thiobutabarbital (Inactin, 100 mg/kg ip; BYK-Gulden, Constance, Germany). Supplemental doses of anesthetic were given as needed to maintain a uniform level of anesthesia. The trachea was cannulated with a polyethylene catheter (PE-240; Intramedic, Clay Adams, Sparks, MD), and the rats either breathed room air or were ventilated with room air enriched with 95% O$_2$-5% CO$_2$ by a Harvard model 683 (South Natick, MD) rodent ventilator at a tidal volume of 2.5 ml at a rate of 30 breaths/min. Polyethylene catheters (PE-50) were inserted into an external jugular vein for the intravenous administration of drugs and into the carotid artery for the measurement of systemic arterial pressure. Systemic arterial pressure was measured with a Viggo-Spectramed transducer (Oxnard, CA) and was recorded on a Grass model 7 polygraph (Grass Instrument, Quincy, MA). Mean pressure was derived by electronic averaging.

For studies in the hindquarters vascular bed, a 1.0- to 1.5-cm segment of the distal aorta was exposed through a ventral midline incision and cleared of surrounding connective tissue. After administration of heparin sodium (1,000 U/kg iv), the aorta was ligated and catheters were inserted into the aorta proximal and distal to the ligature. Blood was withdrawn from the proximal catheter and pumped at a constant-flow rate with a Masterflex pump (Cole-Parmer Instrument, Chicago, IL) into the distal aortic catheter. Perfusion pressure was measured from a lateral tap in the perfusion circuit between the pump and the distal aortic catheter. Hindquarters perfusion and systemic arterial pressures were measured with Viggo-Spectramed transducers and were recorded on a Grass model 7 polygraph. The pump flow rate was set so that perfusion pressure was -- 125 mmHg and was not changed during an experiment. The perfusion
pump flow rate was calibrated by timed collection of blood from the outlet side of the perfusion pump, and the perfusion rate ranged from 3 to 6 ml/min. Agonists were injected directly into the hindquarters perfusion circuit distal to the pump in small volumes (30–100 µl) in a random sequence. Responses to 3–4 agonists could be investigated in the same animal, and systemic arterial or hindquarters perfusion pressure was allowed to return to control value before the next dose of an agonist was injected. The interval between agonist injections was ~10 min. The hindquarters vascular bed was denervated by ligating and cutting the lumbar sympathetic chain ganglia bilaterally between L3 and L4. The extent of vascular isolation of the hindquarters vascular bed was assessed by measuring pump-off occlusion pressure, which usually approached small vein pressure (10–15 mmHg), indicating low collateral inflow and adequate vascular isolation.

Candesartan (CV-11974, 2-ethoxy-1-[2-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl-1H-benzimidazole-7-carboxylic acid; Astra-Hassle, Molndal, Sweden) was dissolved in a 1 N Na2CO3/0.9% NaCl solution (1:20). Losartan sodium (DUP-753; DuPont-Merck, Wilmington, DE) was dissolved in a 5% NaHCO3-dextrose (50:50) solution. Sodium medofenate, N-α-nitro-arginine methyl ester (L-NAME), angiotensin II, III, and IV, norepinephrine hydrochloride, byramine hydrochloride, α,β-methylene ATP, calcitonin gene-related peptide (CGRP; Sigma Chemical, St. Louis, MO), PD-123,319 (Research Biochemicals, Natick, MA), reserpine phosphate (Serpasil), and phentolamine mesylate (CIBA-GEIGY, Summit, NJ) were dissolved in 0.9% NaCl. U-46619 (Upjohn, Kalamazoo, MI) was dissolved in 100% ethanol at a concentration of 10 mg/ml and was diluted in 0.9% NaCl. BAY K 8644 (Miles, New Haven, CT) was dissolved in a 1:4 solution of cremophor EL, tris(hydroxymethyl)aminomethane (Tris), and Tris·HCl (50 mM, pH 7.4). The resulting suspension was warmed, and polyethylene glycol and Tris (pH 7.4) were added to make a stock solution that was stored in a brown bottle in a freezer. Working solutions of all agonists were prepared on a frequent basis, stored in brown, stoppered glass bottles, and kept on crushed ice throughout the duration of the experiment. The agonists used in these studies were injected intravenously or directly into the hindquarters perfusion circuit. Values for the agonists and antagonists did not alter systemic arterial or hindquarters perfusion pressure or responses to the vasoactive agents.

In the first series of experiments, responses to angiotensin II, III, and IV and the effects of candesartan and PD-123,319 on changes in systemic arterial pressure in response to the angiotensin peptides were investigated. The doses of candesartan used were based on doses used in previous studies and on pilot studies in which it was observed that responses to angiotensin II and III were greatly diminished or abolished by candesartan in intravenous doses of 100–1,000 µg/kg (6, 17, 19, 26). Candesartan has been shown to have high affinity for the AT2 receptor and the dose of PD-123,319 used was based on previous studies in the literature (2, 3, 10, 11, 24). Because changes in total peripheral resistance and in cardiac output contribute to the changes in systemic arterial pressure in response to the angiotensin peptides, vascular responses were further analyzed under conditions of controlled blood flow in the hindlimb vascular bed of the rat, where changes in perfusion pressure directly reflect changes in regional vascular resistance and denervation prevented reflex changes in perfusion pressure.

In experiments in which the role of the adrenergic nervous system in mediating increases in systemic arterial pressure was assessed, the effects of pretreatment with the adrenergic neuronal blocking agent reserpine and treatment with the α-receptor-blocking agent phentolamine were investigated. Pretreatment with reserpine (1.5 mg/kg ip) 24 h before the experiment resulted in a decrease in systemic arterial pressure from 125 ± 4 to 95 ± 5 mmHg compared with values in control animals. Administration of phentolamine in an intravenous dose of 1 mg/kg decreased systemic arterial pressure from 131 ± 6 to 107 ± 5 mmHg. The administration of the cyclooxygenase inhibitor sodium medofenate in an intravenous dose of 2.5 mg/kg had no consistent effect on systemic arterial pressure. The nitric oxide synthase inhibitor L-NAME increased systemic arterial pressure from a baseline value of 122 ± 9 to 209 ± 10 mmHg when administered in an intravenous dose of 50 mg/kg.

The hemodynamic data are expressed in absolute millimeters Hg change from baseline and were analyzed using a one-way analysis of variance and Scheffé’s F-test with a Bonferroni-Dunn procedure or a paired t-test. A P value of <0.05 was used as the criterion for statistical significance.

RESULTS

Analysis of responses to angiotensin II: role of the adrenergic nervous system, the cyclooxygenase pathway, and nitric oxide. Intravenous injections of angiotensin II and of angiotensin III in doses of 0.1–3 µg/kg caused dose-related increases in systemic arterial pressure in the anesthetized rat (Fig. 1). Intravenous injections of angiotensin IV in doses of 10–100 µg/kg induced dose-related increases in systemic arterial pressure, and this fragment was ~100-fold less potent than angiotensin II or III in increasing systemic arterial pressure in the rat (Fig. 1). The role of the adrenergic system in mediating or modulating responses to angiotensin II and III was investigated, and increases in systemic arterial pressure in response to intravenous injections of angiotensin II and III were not altered in animals pretreated with reserpine (1.5 mg/kg ip) 24 h before the experiment or in animals that were treated with the α-receptor-blocking agent phentolamine in an intravenous dose of 1 mg/kg (Table 1). Reserpine pretreatment significantly enhanced thepressor response to intravenous injections of norepinephrine and significantly reduced thepressor response to intravenous injections of tyramine (Table 1). Phentolamine significantly reduced thepressor response to intravenous injections of norepinephrine (Table 1). Increases in systemic arterial pressure in response to angiotensin II and III were not
altered after treatment with the cyclooxygenase inhibitor sodium medofenamate (2.5 mg/kg iv; Table 2). The role of nitric oxide release in modulating pressor responses to angiotensin II and III was investigated, and, after treatment with the nitric oxide synthase inhibitor L-NAME (50 mg/kg iv), pressor responses to angiotensin peptides and norepinephrine were increased significantly (Table 2).

Role of AT1 and AT2 receptors. The role of AT1 and AT2 receptors in mediating or modulating pressor responses to angiotensin II and III was investigated, and candesartan and PD-123,319 in the doses used had no significant effect on baseline systemic arterial pressure when values were compared before and 10–20 min after administration of candesartan in intravenous doses of 1 and 10 mg/kg and PD-123,319 in a dose of 10 mg/kg (Table 3). The increases in systemic arterial pressure in response to intravenous injections of angiotensin II and III were reduced significantly after administration of the AT1 receptor antagonist candesartan in an intravenous dose of 1 mg/kg, and there was little tendency for the blockade to be surmounted or overcome when doses of angiotensin II up to 1,000 µg/kg were injected intravenously after administration of the AT1 receptor antagonist (Fig. 1). The AT1 receptor blockade induced by candesartan was long in duration, and pressor responses to angiotensin II were markedly attenuated for periods up to 3 h after administration of the AT1 receptor antagonist (Fig. 2). Although pressor responses to angiotensin II were markedly suppressed for periods up to 3 h after administration of the AT1 receptor antagonist, increases in systemic arterial pressure in response to intravenous injections of norepinephrine were not altered during this same time period (Fig. 2).

The role of the AT2 receptor in mediating or modulating pressor responses to angiotensin II and III was investigated, and these data are summarized in Fig. 3. Increases in systemic arterial pressure in response to angiotensin II and III were not changed after administration of the AT2 receptor antagonist PD-123,319 in an intravenous dose of 10 mg/kg (Fig. 3). The subsequent administration of candesartan (1 mg/kg iv) significantly attenuated pressor responses to angiotensin II and III (Fig. 3). PD-123,319, in an intravenous dose of 10 mg/kg, had no significant effect on the increase in systemic arterial pressure in response to intravenous injections of angiotensin IV (Fig. 3). However, subsequent administration of candesartan (1 mg/kg iv) significantly attenuated the pressor response to angiotensin IV (Fig. 3). Increases in systemic arterial pressure in

Table 1. Influence of reserpine (1.5 mg/kg ip) and phentolamine (1 mg/kg iv) on pressor responses in the rat

<table>
<thead>
<tr>
<th>Increase in Systemic Arterial Pressure, mmHg</th>
<th>Control</th>
<th>Reserpine</th>
<th>Control</th>
<th>Phentolamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II 0.3 µg/kg iv</td>
<td>43 ± 7</td>
<td>38 ± 6</td>
<td>46 ± 7</td>
<td>40 ± 8</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td>(n=10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 µg/kg iv</td>
<td>54 ± 9</td>
<td>47 ± 8</td>
<td>60 ± 9</td>
<td>50 ± 7</td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td>(n=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin III 0.3 µg/kg iv</td>
<td>39 ± 5</td>
<td>32 ± 7</td>
<td>42 ± 7</td>
<td>37 ± 4</td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td>(n=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 µg/kg iv</td>
<td>44 ± 7</td>
<td>40 ± 8</td>
<td>48 ± 6</td>
<td>43 ± 7</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td>(n=10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine 1.0 µg/kg iv</td>
<td>26 ± 4</td>
<td>72 ± 9*</td>
<td>31 ± 6</td>
<td>16 ± 3*</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td>(n=10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyramine 300 µg/kg iv</td>
<td>42 ± 5</td>
<td>5 ± 2*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td>(n=9)</td>
<td></td>
<td></td>
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</tbody>
</table>

Values are means ± SE. n, Number of animals in an experimental group. *Significantly different from control (P < 0.05); paired t-test; ND, not determined.

Table 2. Influence of L-NAME (50 mg/kg iv) and sodium medofenamate (2.5 mg/kg iv) on pressor responses in the rat

<table>
<thead>
<tr>
<th>Increase in Systemic Arterial Pressure, mmHg</th>
<th>Control</th>
<th>L-NAME</th>
<th>Control</th>
<th>Medofenamate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II 0.3 µg/kg iv</td>
<td>46 ± 4</td>
<td>58 ± 7*</td>
<td>51 ± 7</td>
<td>46 ± 8</td>
</tr>
<tr>
<td>(n=4)</td>
<td></td>
<td>(n=4)</td>
<td>(n=6)</td>
<td></td>
</tr>
<tr>
<td>1.0 µg/kg iv</td>
<td>57 ± 6</td>
<td>72 ± 10*</td>
<td>57 ± 10</td>
<td>50 ± 8</td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td>(n=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin III 0.3 µg/kg iv</td>
<td>40 ± 5</td>
<td>56 ± 6*</td>
<td>47 ± 7</td>
<td>42 ± 6</td>
</tr>
<tr>
<td>(n=4)</td>
<td></td>
<td>(n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 µg/kg iv</td>
<td>32 ± 6</td>
<td>53 ± 8*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>(n=4)</td>
<td></td>
<td>(n=4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. L-NAME, N-nitro-L-arginine methyl ester. *Significantly different from control (P < 0.05); paired t-test.
response to norepinephrine were not altered by PD-123,319 or by PD-123,319 and candesartan (Fig. 3).

Analysis of responses to angiotensin peptides in the hindquarters vascular bed. Under constant-flow conditions, injections of angiotensin II, III, and IV into the perfusion circuit induced dose-related increases in hindquarters perfusion pressure (Fig. 4). The effects of candesartan on hindquarters vasoconstrictor responses to the angiotensin peptides were investigated, and these data are also summarized in Fig. 4. After the administration of candesartan in an intravenous dose of 1 mg/kg, increases in hindquarters perfusion pressure in response to angiotensin II, III, and IV were significantly decreased (Fig. 4). Responses to higher doses of angiotensin II were evaluated after administration of candesartan, and the AT1 receptor blockade was not surmounted or overcome when doses of angiotensin II up to 100 µg were injected into the hindquarters perfusion circuit (Fig. 4). Vasoconstrictor responses to norepinephrine were not altered after administration of candesartan in an intravenous dose of 1 mg/kg (Fig. 4). The administration of the AT2 receptor antagonist PD-123,319 in an intravenous dose of 10 mg/kg had no significant effect on increases in hindquarters perfusion pressure in response to angiotensin II or III (Fig. 5). However, the subsequent administration of candesartan in these animals in a dose of 1 mg/kg significantly decreased hindquarters vasoconstrictor responses to angiotensin II and III (Fig. 5).

Selectivity studies. The effects of candesartan on vasoconstrictor responses to U-46619, α,β-methylene ATP, and BAY K 8644 and on vasodilator responses to CGRP were investigated in the hindquarters vascular bed of the rat, and these data are summarized in Fig. 6. After administration of candesartan in an intravenous dose of 1 mg/kg, increases in hindquarters perfusion pressure in response to U-46619, α,β-methylene ATP,
and BAY K 8644 and decreases in perfusion pressure in response to CGRP were not altered (Fig. 6).

Effects of a larger dose of candesartan and of losartan. The effects of a larger dose of candesartan and of losartan on responses to angiotensin II and III were investigated to determine if a vasodepressor or vasodilator response could be unmasked, and these results are summarized in Figs. 7 and 8. After administration of candesartan in an intravenous dose of 10 µg/kg, increases in systemic arterial pressure in response to intravenous injections of angiotensin II and III were reduced significantly (Fig. 9). Pressor responses to intravenous injections of norepinephrine were not changed after administration of candesartan (10 µg/kg iv) (data not shown).

DISCUSSION

The present results show that angiotensin II and III increase systemic arterial and hindquarters perfusion pressures in a dose-related manner in the rat. The increases in systemic arterial and hindquarters perfusion pressure in response to angiotensins II and III were markedly inhibited or abolished by candesartan, an angiotensin AT_1 receptor antagonist. The AT_1 receptor blockade was not surmounted or overcome when larger doses of angiotensin II were injected after administration of candesartan, and the dose-response curves for angiotensin II and III were shifted to the right in a nonparallel manner and exhibited very little positive slope. Although responses to angiotensin II and III were markedly attenuated, candesartan had no signifi-

The effects of a lower dose of candesartan on responses to the angiotensin peptides were investigated, and these data are summarized in Fig. 9. After administration of candesartan in an intravenous dose of 10 µg/kg, increases in systemic arterial pressure in response to intravenous injections of angiotensins II and III were reduced significantly (Fig. 9). Pressor responses to intravenous injections of norepinephrine were not changed after administration of candesartan (10 µg/kg iv) (data not shown).
cant effect on increases in systemic arterial or hindquarters perfusion pressure in response to norepinephrine. The selectivity of the inhibitory effects of candesartan was further studied in the hindquarters vascular bed; and, in addition to not altering responses to norepinephrine, the AT_1 receptor antagonist in a dose (1 mg/kg iv) that markedly inhibited responses to the angiotensin peptides had no effect on vasoconstrictor responses to U-46619, α,β-methylene ATP, and the calcium channel opener BAY K 8644 or on vasodilator responses to CGRP. The inhibitory effect of candesartan on pressor responses to the angiotensin peptides was long in duration with little tendency for responses to return toward control value at periods up to 3 h after administration of candesartan in a dose of 1 mg/kg iv. n, Number of experiments. *Significantly different than control (P < 0.05, ANOVA).

Although increases in systemic arterial and hindquarters perfusion pressure in response to angiotensin II, III, and IV were attenuated or abolished by candesartan, a hypotensive or vasodilator response was not observed after administration of the AT_1 receptor antagonist. These data suggest that vasoconstriction is the predominant response to the angiotensin peptides, and that this response is mediated by the activation of AT_1 receptors in the rat. The present data indicate that blockade of the AT_1 receptor with candesartan or losartan does not unmask a vasodilator response. Moreover, when the dose of candesartan was increased to 10 mg/kg iv, increases in systemic arterial and hindquarters perfusion pressures in response to angiotensin II and III were abolished; and a vasodepressor or vasodilator response was not observed. It is possible that at

Fig. 6. Effect of candesartan on increases in hindquarters perfusion pressure in response to the thromboxane A_2 mimic U-46619, the purinergic P_2x receptor agonist α,β-methylene ATP, and the calcium channel opener BAY K 8644 and on decreases in perfusion pressure in response to calcitonin gene-related peptide (CGRP). Responses were determined before and beginning 10 min after administration of candesartan in a dose of 1 mg/kg iv. n, Number of experiments. *Significantly different than control (P < 0.05, ANOVA).

Fig. 7. Effect of a large dose of candesartan (10 mg/kg iv) on increases in systemic arterial pressure in response to iv injections of angiotensin II and III in 1 group of animals (left panels) and on increases in hindquarters perfusion pressure in response to ia injections of angiotensin II and III in a second group of animals (right panels). Responses to angiotensin peptides were determined before and beginning 10 min after administration of candesartan in a dose of 10 mg/kg iv. n, Number of animals. *Significantly different from control (P < 0.05, ANOVA).
high doses candesartan may also block AT\textsubscript{2} receptors; however, in a manner similar to that observed with candesartan, responses to angiotensin II or III were not reversed by losartan in the present study. The increases in systemic arterial pressure in response to angiotensin II, III, and IV were not altered by the AT\textsubscript{2} receptor antagonist PD-123,319, whereas administration of candesartan to the same animals inhibited pressor responses to the angiotensin peptides. Increases in hindquarters perfusion pressure in response to angiotensin II and III were not altered by PD-123,319. These data are consistent with previous studies in the regional vascular bed of the cat and provide support for the hypothesis that increases in systemic arterial and hindquarters perfusion pressure in response to the angiotensin peptides are mediated by the activation of AT\textsubscript{1} receptors and that AT\textsubscript{2} receptors play little, if any, role in modulating these responses in the rat (5, 6). The present data with PD-123,319 are consistent with results of studies with the AT\textsubscript{2} receptor antagonists PD-123,177 in the rat and PD-123,319 in the regional vascular bed of the cat (5, 6, 8, 9, 16, 29, 31). However, the efficacy of the AT\textsubscript{2} receptor blockade induced by PD-123,317 or PD-123,319 cannot be confirmed in in vivo studies, since a selective AT\textsubscript{2} receptor agonist is not available at the present time.

It has been reported that angiotensin II and III induce biphasic changes in systemic arterial pressure in the anesthetized rat, and that losartan eliminated the pressor response and enhanced the depressor response (24). In the present studies, however, biphasic changes in pressure in response to the angiotensin peptides were almost never observed and responses to angiotensin II and III were not enhanced by the AT\textsubscript{2} receptor antagonist PD-123,319. These data indicate that angiotensin peptides do not induce vasodilation in the hindquarters vascular bed of the rat, that increases in systemic arterial and hindquarters perfusion pressure in response to the peptides were attenuated or abolished but were not reversed by candesartan in doses of 1 and 10 mg/kg iv, and that similar results were obtained with the competitive AT\textsubscript{1} antagonist losartan. These data suggest that AT\textsubscript{2} receptors do not mediate a vasodilator response or modulate pressor responses to the angiotensin peptides in the systemic and hindquarters vascular beds in the rat and are in agreement with previous studies (5, 6, 15, 16, 20). However, the efficacy and selectivity of the AT\textsubscript{2} receptor blockade must be clarified in future studies when a selective AT\textsubscript{2} receptor agonist is developed.

The reason for the difference in results in the present study and in previous studies in the rat is uncertain and does not involve differences in anesthetic, since similar data were obtained with pentobarbital or thiobu-

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**Fig. 8.** Top: influence of losartan (DUP-753; 10 mg/kg iv) on increases in systemic arterial pressure in response to iv injections of angiotensin II and angiotensin III in 1 group of animals. Bottom: influence of PD-123,319 (10 mg/kg iv) and subsequent administration of losartan (10 mg/kg iv) on increases in systemic arterial pressure in response to iv injections of angiotensin II and III in a second group of animals. *Significantly different from control (P < 0.05, ANOVA).

*E121 RESPONSES TO ANGIOTENSIN IN THE RAT*
The effects of the cyclooxygenase inhibitor sodium mesylate on responses are modulated by the release of endothelial tensin peptides act on the endothelium and that regulatory nervous system are not dependent on an interaction with the adrenergic nervous system. The role of the adrenergic nervous system in mediating pressor responses to the angiotensin peptides in the rat was investigated and, after treatment with the adrenergic neuronal blocking agent reserpine, responses to intravenous injections of the angiotensin peptides were not changed. Pretreatment with reserpine decreased the pressor response to the indirect-acting agonist tyramine and enhanced the response to norepinephrine, indicating that adrenergic nerve terminal function was impaired. The effect of the α-receptor blocking agent phentolamine and reserpine on responses to the angiotensin peptides was similar. Treatment with phentolamine in a dose that reduced the pressor response to norepinephrine was without effect on pressor responses to angiotensin II and III. The results with reserpine and with phentolamine indicate that, under the conditions of the present experiments, the increases in systemic arterial pressure in response to intravenous injections of angiotensin II and III, which are mediated by the activation of AT1 receptors, are not dependent on an interaction with the adrenergic nervous system.

In addition to having an interaction with the adrenergic nervous system, it has been postulated that angiotensin peptides act on the endothelium and that responses are modulated by the release of endothelial factors. To ascertain the role of endothelial factors, the effects of the cyclooxygenase inhibitor sodium medofenamate and of the nitric oxide synthase inhibitor L-NAME on responses to the angiotensin peptides were investigated. The results with medofenamate in a dose that attenuates responses to the prostaglandin precursor, arachidonic acid, suggest that increases in systemic arterial pressure in response to intravenous injections of angiotensin II and III are not modulated by the release of vasodilator products in the cyclooxygenase pathway. Nitric oxide is released from the endothelium and could modulate pressor responses to the angiotensin peptides. The results of the present study with L-NAME show that pressor responses to angiotensin II and III are increased significantly. However, this effect is not specific for angiotensin peptides in that pressor responses to norepinephrine were also increased to a similar extent. The results of these experiments when taken together suggest that responses to the angiotensin peptides are mediated by the activation of AT1 receptors on vascular smooth muscle and that the release of norepinephrine, vasodilator prostaglandins, or the activation of AT2 receptors does not play an important role in mediating or modulating the pressor response. It should, however, be pointed out that in vivo AT2 receptor blocking properties of PD-123,319 require further study. The results with L-NAME suggest, however, that pressor responsiveness may be modulated by the release of nitric oxide from the endothelium.

In conclusion, the results of the present study show that AT1 receptor antagonists inhibit responses to angiotensin II, III, and IV in the systemic and hindquarters vascular bed in the rat. These data suggest that the antagonism induced by candesartan in intravenous doses of 1 and 10 mg/kg is noncompetitive in nature and selective and that pressor responses to the angiotensin peptides are not reversed by AT1 receptor antagonists in the rat. The AT2 receptor antagonist PD-123,319 did not alter responses to the angiotensin peptides in the systemic or hindquarters vascular bed of the rat. The present data suggest that the predominant response to the angiotensin peptides is an AT1 receptor-mediated pressor response that is not dependent on an interaction with the adrenergic nervous system and that AT2 receptors or the release of cyclooxygenase products does not appear to play an important role in modulating pressor responses to the angiotensin peptides in the anesthetized rat.

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