Distribution and Metabolism of Angiotensin I and II in the Blood Vessel Wall

Peter Gohlke, Peter Bunning, and Thomas Unger

The demonstration of all components of the renin-angiotensin system in vascular tissue has raised questions as to the precise location of the local angiotensin II generation within the vascular wall. We investigated the metabolism of angiotensin I to angiotensin II in the vascular wall in the isolated rabbit thoracic aorta. Angiotensin I (3×10⁻⁸ M) applied into the aortic lumen was partially converted to angiotensin II (14% after 60 minutes), but most of the luminal angiotensin I was degraded to peptide fragments or diffused as intact angiotensin I, peptide fragments, or both, into the vessel wall. Incubation studies with [³H]angiotensin I revealed that angiotensin I or angiotensin I fragments mainly diffused into the medial layer of the aorta and to a lesser degree into the adventitia and the endothelium. After removal of the endothelium, angiotensin II generation could no longer be detected. Addition of the angiotensin converting enzyme inhibitor ramiprilat (10⁻⁷ M) to the incubation medium led to a complete blockade of angiotensin II generation by endothelial angiotensin converting enzyme. Our results underline the importance of the endothelium for conversion of angiotensin I to angiotensin II and provide evidence that conversion of angiotensin I to angiotensin II is predominantly achieved by endothelial cells. They also support the concept of an endocrine versus autocrine/paracrine renin-angiotensin system where the endothelium of the vasculature is the critical target site for angiotensin II production by both systems and, thus, the most important site for the actions of angiotensin converting enzyme inhibitors. (Hypertension 1992;20:151-157)

KEY WORDS • angiotensin I • angiotensin II • kininase II • angiotensin converting enzyme inhibitors • endothelium, vascular • renin-angiotensin system • rabbit studies

Biochemical, immunohistochemical, and molecular biological studies have provided evidence for the presence of all the components of the renin-angiotensin system (RAS) (renin, angiotensinogen, angiotensin converting enzyme (ACE), angiotensin peptides, and angiotensin receptors) in a variety of tissues, suggesting the existence of local tissue RAS. In addition, local production of angiotensin I (Ang I) and angiotensin II (Ang II) has been demonstrated in cell culture and in organ preparations, and evidence that such a local Ang II production may also occur in humans has been found in human subjects in vivo. However, a precise characterization of a local vascular RAS is complicated by the lack of information about the origin and location of each component of the vascular RAS. Ang I may be synthesized locally within the vascular wall (e.g., medial and adventitial layer) by the action of vascular renin on vascular angiotensinogen, both derived from either local production or systemic uptake. However, the last step in the RAS cascade, i.e., the conversion of Ang I to Ang II, still remains controversial with regard to the site of Ang II production as well as the enzymes involved.

Theoretically, several Ang II-generating pathways can participate in vascular Ang II production. Ang I may be converted to Ang II by endothelial ACE, extracellular ACE, or by other enzymes in the vascular wall like the chymostatin-sensitive Ang II generating enzyme. Furthermore, alternative pathways for Ang I conversion to Ang II, for example, a consecutive action of carboxypeptidases to generate Ang II, may be involved in vascular Ang II production. Moreover, Ang I is not only metabolized to Ang II but also to peptide fragments by the action of aminopeptidases, carboxypeptidases, and endopeptidases localized on vascular endothelial and smooth muscle cells.

In the present study we used an in vitro model, the isolated thoracic rabbit aorta, to investigate the vascular metabolism of Ang I to Ang II and to further analyze the distribution of these peptides and their fragments in the vascular wall.

**Methods**

**Aortic Preparation**

Male Chinchilla rabbits (Möllegard, Skensved, Denmark) weighing 2–3 kg were killed by cervical dislocation. The thoracic aorta was removed and rinsed with a modified Krebs-Henseleit buffer, pH 7.4 (mM: NaCl...
Angiotensin incubation was used for an additional incubation period of 90 minutes. In a second experiment, aortic vessels (n=3) were incubated with $3 \times 10^{-9}$ M Ang I together with the ACE inhibitor ramiprilat ($10^{-7}$ M) and incubated for 5, 10, 15, 30, 45, and 60 minutes. Each incubation period was followed by a washout period of 10 minutes with buffer containing $10^{-7}$ M ramiprilat. In a third experiment, endothelium-denuded aortas (n=3) were incubated with $3 \times 10^{-9}$ M Ang I for periods of 5, 10, 15, 30, 45, and 60 minutes. In a fourth set of experiments, aortic vessels (n=3) were incubated with $10^{-8}$ M Ang II (n=3) for the same incubation periods. Incubations were terminated by sampling the luminal fluid.

Aliquots of 200 µl were added to 50 µl of 0.5 M HCl and stored at -20°C. The concentrations of Ang I and immunoreactive Ang II (irAng II) were determined by radioimmunoassay (RIA) as described previously. Cross-reactivity of the Ang II antibody is 100% for angiotensin III, Ang II-(3-8), and Ang II-(4-8) and 0.5% for Ang I. The cross-reactivity of the Ang I antibody is 100% for Ang I-(2-10) and less than 0.1% for Ang II.

**Distribution of Angiotensin I and Angiotensin II in the Vascular Wall**

The time-dependent distribution of Ang I and Ang II in the vascular wall was investigated by instilling tritium-labeled Ang I or Ang II into the aortic lumen. [Tyr3,3H(N)]-Ang I (0.54 mCi/ml; 33.67 Ci/mmol; custom synthesis) and [Tyr3-5,3H(Thr)]-Ang II (1 mCi/ml; 86.3 Ci/mmol) were obtained from New England Nuclear, Du Pont de Nemour, Boston, Mass. [3H]Ang I or [3H]Ang II at a concentration of $10^{-9}$ M was instilled into the aortic lumen and incubated for 5, 10, 15, 30, 45, 60, and 90 minutes. One aorta was used for each time period of incubation. At the end of the incubation period, radioactivity was measured in the luminal fluid; the endothelial, medial, and adventitial layer; and in the incubation bath. The vascular layers were separated mechanically and transferred into a porcelain vessel for catalytic combustion in the oxygen stream of a combustion furnace (OX 300, Zinsser Analytics, Frankfurt, FRG). This procedure resulted in the generation of tritium, which was rinsed together with scintillator fluid (Quicksint 212, Zinsser Analytics) into a vial (Zinsser Analytics). The recovery was determined using a known amount of radioactivity with and without catalytic combustion. Radioactivity was measured in $\beta$-scintillation counter (2000 CA Tricarb, Packard Instruments Co., Inc., Meriden, Conn.) and expressed as disintegrations per minute (dpm).

**Results**

**Angiotensin Incubation**

Ang I decreased time-dependently in the aortic lumen, whereas irAng II appeared after 5 minutes in the lumen and increased with time throughout the incubation period (Table 1, Figure 2A). After 60 minutes of incubation the intraluminal Ang I concentration had decreased by 73%. At this time point, the irAng II concentration in the lumen reached 14% of the initial concentration of Ang I. Addition of the ACE inhibitor ramiprilat ($10^{-7}$ M) into the incubation medium reduced intraluminal irAng II concentration to virtually zero (Figure 2B). The decrease of the intraluminal Ang...
### TABLE 1. Intraluminal Incubation of Angiotensin I in Rabbit Aorta

<table>
<thead>
<tr>
<th>Incubation period (min)</th>
<th>Ang I (pg/50 µl)</th>
<th>Ang II (pg/50 µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>239.2±40.7</td>
<td>...</td>
</tr>
<tr>
<td>5</td>
<td>219.3±26.7</td>
<td>7.7±1.6</td>
</tr>
<tr>
<td>10</td>
<td>204.2±56.0</td>
<td>12.4±3.2</td>
</tr>
<tr>
<td>15</td>
<td>152.3±31.4</td>
<td>18.4±2.6</td>
</tr>
<tr>
<td>30</td>
<td>111.3±23.4</td>
<td>20.5±3.6</td>
</tr>
<tr>
<td>45</td>
<td>98.2±24.6</td>
<td>28.8±9.2</td>
</tr>
<tr>
<td>60</td>
<td>60.3±15.8</td>
<td>31.5±5.6</td>
</tr>
</tbody>
</table>

Intraluminal angiotensin I (Ang I) and angiotensin II (Ang II) were measured by radioimmunoassay after different incubation periods. Values are expressed as mean±SEM (n=6). Time zero represents the initial intraluminal administered concentration of Ang I.

I concentration was initially delayed, but after 60 minutes of incubation a reduction similar to that in untreated aortas was observed (69% with ramiprilat versus 73% without ramiprilat). Removal of the endothelium prevented the irAng II generation without affecting Ang I decrease in the aortic lumen (Figure 2C). Administration of Ang II into the aortic lumen revealed kinetics similar to Ang I (71% decrease after 60 minutes of incubation) (Figure 3).

The histological examination of the aortic luminal surface demonstrated the integrity of the endothelium before (Figure 4A) and after (Figure 4B) consecutive angiotensin incubations. The endothelium remained intact with some small scattered lesions after consecutive angiotensin incubations. The completeness of the removal of the endothelium was also verified histologically (Figure 4C). Only smooth muscle cells of the vascular media could be detected in the denuded aortic vessels.

### Distribution of Angiotensin I and Angiotensin II in the Vascular Wall

The time-dependent distribution of radioactivity in the luminal fluid, the vascular wall, and the incubation bath after instillation of [3H]Ang I into the aortic lumen is presented in Figure 5. Radioactivity in the vessel lumen, representing Ang I, Ang I fragments, or both, decreased with time in a linear fashion. After 60 minutes' and 90 minutes' incubation, 37% and 46% of the initial amount of radioactivity had diffused into the vascular wall, mainly into the medial layer (25% after 60 minutes and 31% after 90 minutes). Only small amounts of radioactivity were measured in the endothelial and adventitial layers (Figure 5).

Radioactivity in the incubation bath, reflecting penetration of Ang I or its fragments, or both, through all vascular layers, remained low up to 30 minutes and then increased to 8.2% and 8.6% after 60 minutes and 90 minutes, respectively.

The distribution of [3H]Ang II in the vascular wall is shown in Figure 6. The results were comparable to those for [3H]Ang I, except for higher levels of radioactivity in the incubation bath after 60 and 90 minutes (14% and 16.5%, respectively). The fate of Ang I in the aortic wall was summarized from both experiments. For each incubation time we added the percentage amount of Ang I left in the aortic lumen, the percentage amount of irAng II generated within the lumen, and the percentage amount of radioactivity that had diffused out of the lumen. The difference between the sum of these three parameters and the initial amount of Ang I (100%) provides a measure for Ang I degradation products that were not detected in the Ang I or Ang II RIA. The results demonstrate...
that Ang I degradation products increased up to 30 minutes of incubation and remained constant thereafter, indicating a steady state between degradation and diffusion (Figure 7).

**Discussion**

The isolated thoracic rabbit aorta represents a valuable model for studies on the metabolism and distribution of angiotensin peptides in the vascular wall. Incubation of intact aortic vessels with Ang I may be particularly suited to mimicking the fate of circulating Ang I in vivo, and after removal of the endothelium, metabolic processes in deeper layers of the vascular wall can be studied. The lack of a pulsatile perfusion inherent to this model has to be acknowledged. However, this drawback is outweighed by the stability and reproducibility of the system and can be accepted if one limits the investigation to general features of peptide handling by the vascular wall.

Incubation of aortic vessels with Ang I for 60 minutes resulted in a 14% conversion of Ang I to Ang II by endothelial ACE. Since an unknown proportion of newly generated Ang II might have been enzymatically degraded or could have penetrated into the vascular wall, or both, the actual generation of Ang II might have been even greater in view of the fact that the incubation of aortic vessels with Ang II revealed a similar "escape" of Ang II, its fragments, or both, into the vascular wall as observed with Ang I.

Our results confirm previous results by Bunning et al., who determined a conversion rate of Ang I to Ang II of 10–20% after incubation with a high concentration of 5 × 10⁻⁷ M Ang I using two in vitro systems, the isolated thoracic rabbit aorta and cultured endothelial cells from rabbit aorta. Similar conversion rates were reported in dog kidney (4–19%), in dog mesenteric arteries (25%), and in the isolated perfused rat heart (7%).

Our data further demonstrate that the major portion of luminal Ang I diffused into the vessel wall or was degraded to peptide fragments. Incubation studies with radioactively labeled Ang I revealed that Ang I, Ang I fragments, or both, accumulated mainly in the medial layer of the vascular wall. This raises the question of a possible conversion of Ang I to Ang II within the medial layer. Generation of Ang I in the vascular media is possible since renin and angiotensinogen both were shown to be present in this layer of the vascular wall and were either synthesized locally or taken up from plasma. However, it is still controversial whether the last step in the RAS cascade, the generation of Ang II, occurs in the vascular media, since the precise location of ACE within the vascular wall is by no means clear. In a study by Velletri and Bean, ACE activity was measured in the vascular wall using a radioenzymatic technique. The authors demonstrated that 42% of the ACE activity was localized in the medial layer while 58% was found in the adventitial layer. A major drawback of this study, however, is the lack of ACE activity in the intimal layer. In another study by Pipili et al., ACE activity was measured in intact as well as in endothelium-denuded aortic rings. Although this study suggested that ACE was present in extracellular sites, the exact localization of ACE, e.g., medial or adventitial layer, or both, still remains obscure. Indirect evidence for an extracellular conversion of Ang I to Ang II in the vascular wall was provided by studies measuring the contraction
of endothelium-denuded aortic rings after addition of Ang I in rabbit aorta and in rat aorta. In these studies aortic rings, suspended in an organ bath, were used for the measurement of isometric contraction after exogenous addition of Ang I. With this model, the medial as well as the adventitial site of the vascular wall is exposed to the peptide. Thus, it is possible that conversion of Ang I to Ang II occurs primarily in the adventitial layer (e.g., vasa vasorum). In contrast, in our model of the isolated thoracic rabbit aorta, Ang I was applied intraluminally and thus was exposed to endothelial enzymes or, after removal of the endothelium, to enzymes on vascular smooth muscle cells.

In the present study, removal of the aortic endothelium completely abolished the conversion of Ang I to Ang II. Thus, there was no evidence for an extraendothelial generation of Ang II in the vascular wall. Our results are supported by a number of studies investigating the localization of ACE in the vascular wall by means of biochemical, immunocytochemical, and autoradiographic methods. Generally, these studies failed to detect any ACE activity in the vascular medial layer, thus pointing to the endothelium as the major site of Ang II generation.

The decrease of luminal Ang I after 1 hour was not different between intact or endothelially denuded aortas, despite the fact that conversion of Ang I to Ang II was abolished in the latter. This finding may be explained either by an increased diffusion rate into the vascular wall due to the absence of the endothelium as diffusion barrier or by an increased degradation of Ang I by peptidases on vascular smooth muscle cells of the medial layer.

Inhibition of the endothelial ACE by addition of the ACE inhibitor ramiprilat (10⁻⁷ M) to the incubation medium resulted in a complete blockade of Ang II generation. However, despite the lack of Ang I conversion to Ang II, the ACE inhibitor did not affect the Ang I decrease in the aortic lumen during an incubation of 1 hour. A compensatorily increased degradation of Ang I by other endothelial peptidases may account for this finding. Similar results were obtained by Lückhoff et al, who investigated the effects of enalapril (10⁻⁷ M) on the metabolism of Ang I (5x10⁻⁷ M) in the isolated rabbit aorta. In contrast, Whalley observed a significant attenuation of the Ang I breakdown by rabbit aorta and human basilar artery in the presence of captopril (5x10⁻⁴ M), although in that study the Ang II production remained unaffected by the ACE inhibitor. These contradictory results may be explained by the very high concentration of Ang I (10⁻⁴ M) used in their study.

In conclusion, our results emphasize the importance of an intact endothelium for vascular Ang II generation and provide evidence that conversion of Ang I to Ang II is predominantly achieved by endothelial cells. They also support the concept of an endocrine versus autocrine/paracrine RAS, where the vascular endothelium is the strategic site for Ang II production for both systems and thus the most important target site for the action of ACE inhibitors.

References
14. Campbell DJ, Habener JF: Cellular localization of angiotensinogen gene expression in brown adipose tissue and mesenteric: Quan-
tification of messenger ribonucleic acid abundance using hybrid-
ization in situ. Endocrinology 1987;121:1616–1626
19. Ahbhold RH, Sullivan MJ, Wright JW, Harding JW: Binding, deg-
radiation and pressor activity of angiotensin II and III after ami-
nopeptidase inhibition with amastatin and bestatin. J Pharmacol Exp Ther 1987;242:957–962
20. Johnsson H, Drummer OH: Hydrolysis of angiotensin I by pepti-


Distribution and metabolism of angiotensin I and II in the blood vessel wall.
P Gohlke, P Bünning and T Unger

Hypertension. 1992;20:151-157
doi: 10.1161/01.HYP.20.2.151

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/20/2/151

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/