Review

Local Angiotensin II-Generating System in Vascular Tissues: the Roles of Chymase

Mizuo MIYAZAKI and Shinji TAKAI

Roles of each angiotensin II producing enzymes of each of the angiotensin II-producing enzymes were reviewed based on experimental models. In vascular tissues, angiotensin II is potentially cleaved from angiotensin I by angiotensin converting enzyme (ACE) and chymase. It has been confirmed that vascular tissues of humans, monkeys, dogs and hamsters have a chymase-dependent angiotensin II-forming pathway. Much like other hypertensive models, hamster hypertensive models show high levels of vascular ACE activity, but not chymase activity. In hypertensive hamsters, administration of either an ACE inhibitor or an angiotensin II type 1 (AT₁) receptor antagonist resulted in similar reductions in blood pressure, suggesting that chymase is not involved in the maintenance of high blood pressure in this model. In monkeys fed a high-cholesterol diet, ACE activity was increased in the atherosclerotic lesions, and an ACE inhibitor and an AT₁ receptor antagonist prevented atherosclerosis to a similar degree, suggesting that ACE may be mainly involved in the development of atherosclerosis. After balloon injury in dog vessels, both ACE and chymase activities were locally increased about 3-fold in the injured arteries, and an AT₁ receptor antagonist was effective in preventing the intimal formation, but an ACE inhibitor was ineffective. In dog grafted veins, the activities of chymase were increased 15-fold, but those of ACE were increased only 2-fold, and the intimal formation was suppressed by either an AT₁ receptor antagonist or a chymase inhibitor. In the normal vascular tissues, ACE plays a crucial role for angiotensin II production, whereas chymase is stored in mast cells in an inactive form. Chymase acquires the ability to form angiotensin II following mast cells activation followed by mast cells activation by a strong stimulus such as occurs in cathereter-injury or grafting. Together, these results indicate that chymase plays a major role in the vascular angiotensin II-generating system, particularly in cases of vascular injury.

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Key Words: angiotensin II, angiotensin-converting enzyme, chymase, hypertension, vascular remodeling

Introduction

Angiotensin (Ang) II is a vasoconstricting peptide derived from angiotensinogen by renin and angiotensin-converting enzyme (ACE). The latter is a well-known enzyme for conversion from Ang I to Ang II. However, several other enzymes have also been reported to produce Ang II. Cathepsin D, like renin, converts angiotensinogen to angiotensin I (1). Chymase (2-4), cathepsin G (5), tonin (6), and kallikrein (7), like ACE, can convert Ang I to Ang II. Ang II has been produced from Ang I in the presence of an excess supramaximum dose of ACE inhibitor in extracts from canine vascular tissues, and this Ang II production was inhibited by chymostatin, but not by aprotinin (8). Chymase and cathepsin G, but not tonin and kallikrein, are inhibited by chymostatin, and cathepsin G, tonin and kallikrein, but not chymase, are inhibited by
Aprotinin (5–7). These findings suggest that canine vascular tissues contain only a chymase-like enzyme as a non-ACE Ang II-forming enzyme; in 1984, this enzyme was designated as the chymostatin-sensitive Ang II-generating enzyme (CAGE) (9). CAGE activity has been observed in extracts from human, monkey and hamster vascular tissues (3, 4, 10). In each of these cases, the respective CAGEs were purified and identified as chymase (3, 4, 10). Therefore, vascular tissues in humans, monkeys, dogs and hamsters posses two enzymes, ACE and chymase, as Ang II-forming enzymes. In this review, we introduce the pathophysiological role of Ang II produced by chymase in vascular tissues based on our studies.

Angiotensin II Formation in Vascular Extracts

In the extracts from human arteries, the major products from Ang I were Ang-(1-9) and Ang II (11). In the human vascular extracts, only 8% of the Ang II formation was inhibited by the ACE inhibitor lisinopril, while 95% was inhibited by chymostatin. These findings suggest that over 90% of the Ang II formation in extracts from human vascular tissues is dependent on chymase. However, chymase is stored in secretory granules in mast cells and ordinarily shows no Ang II formation or other enzymatic activity in situ, while chymase is activated immediately after the degranulation (12, 13). Therefore, in the extracts, the extent of chymase-dependent Ang II-forming activity may be overestimated (14, 15). The other product, Ang-(1-9), was inhibited completely by carboxypeptidase inhibitor, indicating that the conversion of Ang I to Ang-(1-9) is likely to be dependent on carboxypeptidase A (16, 17). On the other hand, in the extracts from rat vessels, the major products from angiotensin I were Ang II and Ang-(5-10) (18). The Ang II formation was suppressed by lisinopril, but not by chymostatin, while the Ang-(5-10) formation was completely inhibited by chymostatin. Therefore, it is certain that, in rat vascular tissues, almost all Ang II formation is dependent on ACE — that is, Ang II-forming enzymes other than ACE, including chymase, are hardly observed. On the other hand, Ang II formation is predominantly dependent on chymase in the extracts from human vascular tissues. In this context, it is noteworthy that most cells of rat vascular tissues contain an enzyme that is identified as chymase, but that is not able to produce Ang II. The finding that human chymase, but not rat chymase, can form Ang II from Ang I is attributable to differences in the substrate specificity of these chymases. Using the substrate Ang I, human chymase does cleave the Phe (8)-His (9) bond of Ang I to yield Ang II, while rat chymase cleaves the Tyr (4)-Ile (5) bond to form inactive fragments (19). Monkey, dog and hamster chymases, like human chymase, cleave the Phe (8)-His (9) bond of Ang I to yield Ang II (3, 4, 20).

These species differences in Ang II-production of chymase should be considered in choosing experimental animals for examining the pathogenesis of human vascular diseases in which Ang II may be involved.

Hypertension

Ang II plays an important role in the regulation of blood pressure. However, the renin and ACE activities in plasma were apparently normal or low in the chronic stage of hypertensive rat models, such as spontaneously hypertensive rats (21, 22), one-kidney, one clip hypertensive rats (23) and two-kidney, one clip (2K1C) hypertensive rats and dogs (24, 25). On the other hand, in the chronic stage of all these hypertensive models, in which blood pressure was lowered by an ACE inhibitor, vascular ACE was activated to increase the local production of vascular Ang II (21–25). These findings suggest that the increase of Ang II generated by ACE in vascular tissues plays a crucial role in the pathogenesis of hypertension. To clarify whether chymase is involved in this process, we studied a 2K1C hypertensive hamster model (26). The blood pressure in the 2K1C hamster increased significantly 2 weeks after clipping (acute stage), and remained at a high level until 32 weeks after clipping (chronic stage). Plasma renin activity increased significantly during the acute stage, but returned to the baseline level at the chronic stage. In the chronic stage, the vascular ACE activity, but not the chymase activity, increased significantly, and an ACE inhibitor and an Ang II type 1 (AT1) receptor antagonist showed equipotently hypotensive effects at the acute and chronic stages (26). These findings suggest that vascular chymase is not involved in the pathogenesis of hypertension, while vascular ACE plays an important role in regulating blood pressure.

Atherosclerosis

Macrophages express ACE, and it is thought that Ang II formation is predominantly dependent on ACE in the atherosclerotic area (27). In fact, ACE inhibition has been shown to effectively prevent atherosclerosis in numerous animal models, including rabbits (28), minipigs (29), hamsters (30) and monkeys (31, 32). In atherosclerotic lesions, an ACE inhibitor suppressed Ang II-inducing mRNAs, such as fibronectin and transforming growth factor-β (31). Furthermore, the atherosclerosis-preventive effect of the ACE inhibitor was equal to that of an AT1 receptor antagonist (33). These evidences suggest that ACE may play an important role in the development of atherosclerosis. However, monkey vascular tissues contain Ang II-forming chymase, and the mRNA of this chymase was also significantly increased in monkey atherosclerotic lesions (3). In humans, it has been reported that both the number of activated mast cells (34)
Table 1. Beneficial Effect of ACE Inhibitor, Chymase Inhibitor and AT₁ Receptor Antagonist

<table>
<thead>
<tr>
<th>Vascular enzyme activity</th>
<th>ACE</th>
<th>Chymase</th>
<th>ACE-I</th>
<th>ARB</th>
<th>CHY-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>↑</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Balloon injury</td>
<td>↑</td>
<td>↑</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Graft</td>
<td>↑</td>
<td>↑</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>↑</td>
<td>↑</td>
<td>+</td>
<td>+</td>
<td>?</td>
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</tbody>
</table>


Fig. 1. Proposed pathways responsible for Ang II formation in vascular tissue. In normal state, ACE in endothelial cells converts Ang I to Ang II, whereas chymase in mast cells in an inactive-type and has no enzymatic effects including Ang II-forming ability. In the injured vessels, mast cells are activated by the stimulus and chymase forms Ang II from Ang I.

and the chymase activity (35) are increased in atherosclerotic lesions. Thus we cannot rule out a possible contribution of chymase in human atherosclerosis.

Vascular Proliferation after Balloon Injury

Ang II plays crucial roles in the migration and proliferation of vascular tissues (36, 37). For example, an ACE inhibitor was effective in preventing the proliferation of vascular tissue after balloon injury of vessels in rats (38). Based on these reports, an ACE inhibitor was used for human vascular restenosis after percutaneous transluminal coronary angioplasty, but a negative result was reported (39, 40). Rat vascular tissues only contain ACE as an Ang II-forming enzyme (18), suggesting that vascular Ang II formation by ACE plays a crucial role in tissue proliferation. Such species differences in the effects of ACE inhibitors on neointimal formation after injury may depend on whether or not a given species possesses Ang II-forming chymase in vascular tissue. In dogs, the activities of chymase and ACE in arteries injured by a balloon catheter were increased compared with those in uninjured arteries 1 month after the operation; an AT₁ receptor antagonist was effective in preventing intimal formation of vessels after injury, but an ACE inhibitor was ineffective (41, 42). Moreover, tranilast, a mast cell-stabilizing agent, prevented neointimal formation with suppression of chymase activity, but not ACE activity (43). These evidences suggest that the chymase-dependent angiotensin II formation may contribute to the development of neointimal
formation induced in vessels after injury by a balloon catheter.

Vascular Proliferation in Grafted Vessels

In previous therapeutic studies of stenosis in grafted vessels, an ACE inhibitor was effective in preventing vascular proliferation in rats, but not in baboons (44, 45). Baboon vascular tissues possess a chymase-dependent Ang II producing system, and the different effects of ACE inhibitors in these animals may thus depend on species differences in the Ang II-formation (19). We investigated the effects of an AT1 receptor antagonist and a chymase inhibitor in a dog graft model (46, 47). Dogs underwent right common carotid artery bypass grafting with the ipsilateral external jugular vein. Four weeks after the operation, the intimal area was significantly increased in the grafted veins. The chymase activity in the grafted veins was increased 15-fold compared with that in control veins, and the ACE activity was doubled. An AT1 receptor antagonist and a chymase inhibitor reduced the intimal area in the grafted veins to 25.5% and 36.1%, respectively (46, 47). The Ang II-inducing mRNAs of extracellular matrix components such as collagens I and III and fibronectin were significantly increased in the grafted veins, while no increase of mRNA levels was observed by a chymase inhibitor (47). The fact that a chymase inhibitor prevented the development of vascular proliferation indicates that chymase-produced Ang II plays a significant role in grafted vessels.

Conclusions

Two types of Ang II generating systems having distinct functions are involved in the vascular tissues. In the normal state, vascular ACE regulates local Ang II formation and plays a crucial role in the regulation of blood pressure, whereas chymase is stored in mast cells and shows no Ang II-forming activity. On the other hand, chymase is activated immediately upon release into the extracellular matrix in vascular tissues after mast cells have been activated by a stimulus such as injury by a catheter or grafting of vessels. The chymase-dependent Ang II formation serves only in vascular incidents. Therefore, chymase may be defined as an inflammatory-induced Ang II-forming enzyme in vascular tissues (Fig. 1 and Table 1).

References


