BRAZILIAN JOURNAL OF MEDICAL AND BIOLOGICAL RESEARCH

www.bjournal.com.br

ISSN 0100-879X

Volume 44 (9) 814-965 September 2011

BIOMEDICAL SCIENCES

Braz J Med Biol Res, September 2011, Volume 44(9) 914-919

doi: 10.1590/S0100-879X2011007500093

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The Brazilian Journal of Medical and Biological Research is partially financed by

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Brazilian Journal of Medical and Biological Research (2011) 44: 914-919
ISSN 0100-879X Review

Alternative pathways for angiotensin II generation in the cardiovascular system

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Abstract

The classical renin-angiotensin system (RAS) consists of enzymes and peptides that regulate blood pressure and electrolyte and fluid homeostasis. Angiotensin II (Ang II) is one of the most important and extensively studied components of the RAS. The beneficial effects of angiotensin-converting enzyme (ACE) inhibitors in the treatment of hypertension and heart failure, among other diseases, are well known. However, it has been reported that patients chronically treated with effective doses of these inhibitors do not show suppression of Ang II formation, suggesting the involvement of pathways alternative to ACE in the generation of Ang II. Moreover, the finding that the concentration of Ang II is preserved in the kidney, heart and lungs of mice with an ACE deletion indicates the important role of alternative pathways under basal conditions to maintain the levels of Ang II. Our group has characterized the serine protease elastase-2 as an alternative pathway for Ang II generation from Ang I in rats. A role for elastase-2 in the cardiovascular system was suggested by studies performed in heart and conductance and resistance vessels of normotensive and spontaneously hypertensive rats. This mini-review will highlight the pharmacological aspects of the RAS, emphasizing the role of elastase-2, an alternative pathway for Ang II generation.

Key words: Renin-angiotensin system; Angiotensin II; Rat elastase-2; Alternative pathway; Angiotensin-converting enzyme

Renin-angiotensin system

It is well established that the main end-product of the renin-angiotensin system (RAS), angiotensin II (Ang II), plays a key role in regulating cardiovascular homeostasis, acting both on the regulation of blood volume and peripheral vascular resistance (1). In recent years, however, several studies have shown that the RAS presents a much greater complexity than previously thought. Many peptides that were considered to be inactive metabolites had their biological activity recognized. Angiotensin III (Ang III), which is formed from Ang II by the action of aminopeptidase A, induces aldosterone release (2). Angiotensin IV (Ang IV), formed from Ang III by aminopeptidase N, has an important role in the central nervous system, especially related to memory, and also displays proliferative effects (3). Angiotensin 1-7 (Ang-(1-7)), via activation of the MAS receptor, seems to have effects opposite to those mediated by Ang II. Another carboxypeptidase homologous to angiotensin-converting enzyme (ACE), called ACE-2, was simultaneously identified by two groups (4,5). ACE-2 cleaves Ang I and Ang II to generate Ang-(1-9) and Ang-(1-7), respectively. It is important to note that so far no direct biological activity for the Ang-(1-9) fragment was described, and it is suggested that this peptide can compete with Ang I by binding the active site of ACE, which then generates Ang-(1-7) from Ang-(1-9) (Figure 1).

The effects of the angiotensin peptides are exerted via activation of different angiotensin receptors, such as type 1 (AT1), type 2 (AT2), type 4 (AT4), and MAS (1). AT1 receptors are expressed in the lungs, liver, kidney, heart, blood vessels, brain, adrenal glands, and various endocrine glands (6), and are activated by Ang II. AT1 receptors can also be stimulated by other mediators of the RAS with lower binding affinity, such as Ang III, Ang IV, and Ang-(1-7). AT2 receptors are predominantly expressed in fetal tissues (a situation reversed after birth), and are also expressed in situations of injury. Ang II and Ang-(1-7) are ligands for the AT2 receptors whose activation leads to vasodilation and anti-proliferative effects. AT2-induced effects seem to antagonize the effects induced by AT1 activation (7). Ang IV binds to the AT4 receptor that is located in the brain, heart, lungs, liver, and kidneys, being related to cognitive functions and proliferative effects (8,9). The MAS receptor

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Presented at the XV Simpósio Brasileiro de Fisiologia Cardiovascular, São Paulo, SP, Brazil, February 2-5, 2011.

Received February 9, 2011. Accepted July 8, 2011. Available online July 22, 2011. Published September 16, 2011.

is activated by Ang-(1-7), inducing vasodilation and antiproliferative effects (10).

ACE inhibitors are widely used not only in the treatment of hypertension, but also in other cardiovascular diseases (or diseases related to them) such as heart failure, myocardial infarction, renal failure, and diabetic nephropathy (11). Results of numerous clinical studies (12,13) have established that blockade of the RAS with ACE inhibitors results in significant reduction of mortality in patients with heart failure or post-myocardial infarction. Since the benefits for survival were demonstrated with different ACE inhibitors, and all have common mechanisms, this important effect is attributed to ACE inhibition. Pharmacological studies show that ACE inhibitors may differ in their affinity for tissue ACE, but the clinical significance of this observation remains to be demonstrated (11).

In the last decade, evidence has accumulated showing that Ang II acts as a circulating hormone, and may also be formed locally in various tissues, acting as an autocrine or paracrine hormone and inducing a broad spectrum of effects on the cardiovascular and renal systems (14,15). Although the beneficial effects of the use of ACE inhibitors in the treatment of hypertension and heart failure, among other diseases, are well known, it has been reported that patients chronically treated with effective doses of these inhibitors do not show suppression of Ang II formation (16,17), suggesting the involvement of alternative pathways for Ang II generation. Moreover, the finding that the concentration of Ang II is maintained in the kidney, heart and lungs of mice with ACE deletion (18) indicates an important role of alternative pathways to maintain basal levels of Ang II.

Alternative pathways for Ang II generation

Several studies have shown the involvement of other enzymes, in addition to ACE, in the generation of Ang II (19-21). The first descriptions of an alternative pathway for Ang II formation were reported by Boucher et al. (22) in the submandibular glands of rats, by Cornish et al. (23) in the hamster cheek, and by Trachte and Lefer (24) in the cat cardiac papillary muscle. Cornish et al. (23) demonstrated that the vasoconstrictor response induced by Ang I in blood vessels of the hamster cheek pouch was only partially blocked by ACE inhibitors but completely abolished by Ang II receptor antagonists, leading to the conclusion that this vascular bed converts significant amounts of Ang I to Ang II by a route that does not involve ACE. Cornish et al. (25) also observed the formation of Ang II independently of ACE in the coronary artery of hamsters. Some years later, Okunishi et al. (26) identified an Ang II-generating enzyme in dog mesenteric artery, which was sensitive to chymostatin and insensitive to ACE inhibitors. Urata et al. (27) demonstrated *in vitro* two pathways for the formation of Ang II in homogenates of human heart. These investigators observed that approximately 80% of the total formation of Ang II was associated with the presence of an unknown serine protease, whereas ACE-dependent Ang II generation was only responsible for approximately 11% of total Ang II. This cardiac serine protease was purified and identified as a new member of the chymase family, and was named human heart chymase (28).

Although several enzymes can produce Ang II *in vitro* through cleavage of the Phe^8-His^9 bond of Ang I, their *in vivo* activity cannot be demonstrated. Moreover, some of these enzymes, such as trypsin, chymotrypsin and rodent chymases, also degrade Ang II (29). Data generated from the susceptibility of these different enzymes to protease inhibitors have allowed the classification of Ang II-forming enzymes into three categories (30). The first category corresponds to metallodipeptidyl carboxypeptidase known as ACE. The second category comprises the aprotinin-sensitive serine proteases, such as kallikrein (31), trypsin (30), tonin (22), and cathepsin G (32). The third category includes a group of chymostatin-sensitive serine proteases, such as the chymostatin-sensitive Ang II-generating enzyme found in the dog mesenteric artery (33), human chymase (27,28) and the rat elastase-2 enzyme (34,35). These two chymostatin-sensitive serine proteases, human chymase

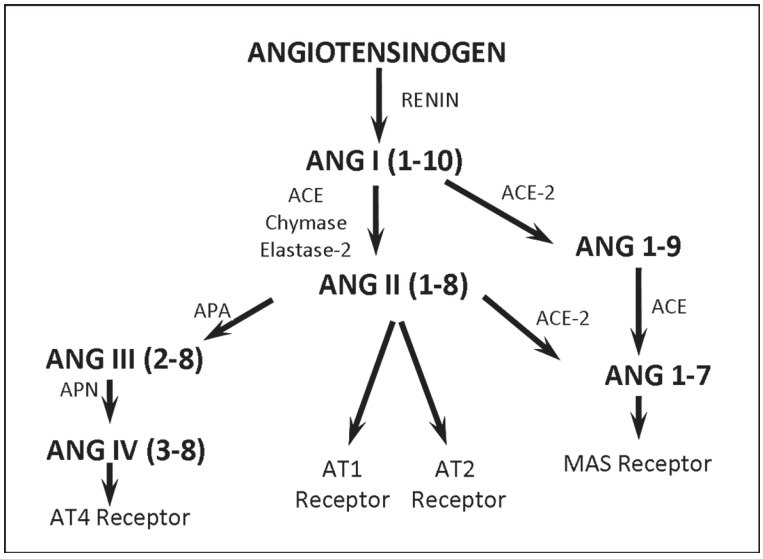

Figure 1. Representative peptides, enzymes, and receptors of the renin-angiotensin system. ANG = angiotensin; ACE = angiotensin-converting enzyme; ACE-2 = angiotensin-converting enzyme-2; APA = aminopeptidase A; APN = aminopeptidase N.

and rat elastase-2, have been well characterized as ACE-independent pathways for Ang II formation in vascular tissue (36-39).

Despite the different characteristics of chymases in different species, these enzymes are functionally relevant in the ACE-independent formation of Ang II (33,40,41). The determination of distinct functional activities for ACE and chymases has been possible in some cases by the use of selective inhibitors (42) and substrates (43). The development of the compound Z-Ile-Glu-Pro-Phe-CO2H (CH5450), a peptide inhibitor of human heart chymase (42), provided an important pharmacological tool to investigate the enzymes responsible for tissue Ang II formation (41). Evidence that enzymatic ACE-independent pathways are functional in the conversion of precursors of circulating Ang II was provided by *in vivo* experiments (43-47) using the synthetic peptide [D-Pro^{11}-Ala^{12}]-Ang I. This biologically inactive Ang II precursor selectively releases Ang II after incubation with human chymase, but not with ACE or carboxypeptidases (43). This substrate has been used as a tool to demonstrate the role of chymases in the formation of Ang II in preparations derived from human tissues (41), primates (43,44), hamsters (46), dogs (48), cats (45), and even rats (47), a species that lacks chymase-dependent Ang II-forming activity.

The chymases are described as an alternative pathway to ACE for Ang II generation. However, depending on the species studied, chymase may be generating or degrading Ang II. Kunori et al. (49) showed that the kinetics of Ang II formation by chymases from different species followed the order dog > human > hamster > mouse > rat (Kcat/km: 18, 11, 0.69, 0.059, 0.0030 mM/min, respectively), whereas the Ang II-degrading activity of chymases is: hamster > rat > mouse > dog (Kcat/km: 5.4, 4.8, 0.39, 0.29 mM/min, respectively), indicating that human chymase has only Ang II-generating activity, whereas rat chymase exhibits Ang II-degrading activity. We have recently described a serine protease, a member of the chymotrypsin-like elastase family, named elastase 2A, which is secreted in the rat mesenteric arterial bed and features Ang II-forming activity (34,38).

Rat elastase-2

We demonstrated the existence of some peptidases in the perfusate of the isolated rat mesenteric bed (50). The recirculating perfusion solution accumulated endo- and exo-soluble peptidases, among which a serine protease insensitive to captopril and capable to form Ang II from Ang I was identified. Later, this Ang II-forming serine protease of the rat mesenteric arterial bed perfusate was isolated and purified by a combination of filtration and affinity chromatography (34). The enzyme was sensitive to chymostatin and was identified as a glycoprotein with a molecular mass of 28.5 kDa. The amino-terminal sequence of the first ten residues of this enzyme was found to be identical to the rat pancreatic elastase-2 (EC 3.4.21.71) and the enzyme was called Ang II-forming elastase-2 of rat mesenteric arterial bed perfusate. The sequencing of cDNAs for pancreatic elastase-2 and rat mesenteric arterial bed showed that they were identical (51). The sequences were also identical to that previously published by MacDonald et al. (52) for elastase-2 from rat pancreas. At present, elastase-2 is the only representative of this family of proteases that is secreted outside the digestive tract and is involved in Ang II generation. The mRNA for elastase-2 was also detected in other tissues, such as lungs, heart, kidney, liver, spleen, and carotid artery (38).

A notable feature of elastase-2 as an Ang I-converting enzyme is that it does not destroy the Ang II product formed by only one cleavage (Figure 2) (34). This characteristic is shared with some chymases, such as human (27), baboon (43), and dog (53) chymases, but not with the

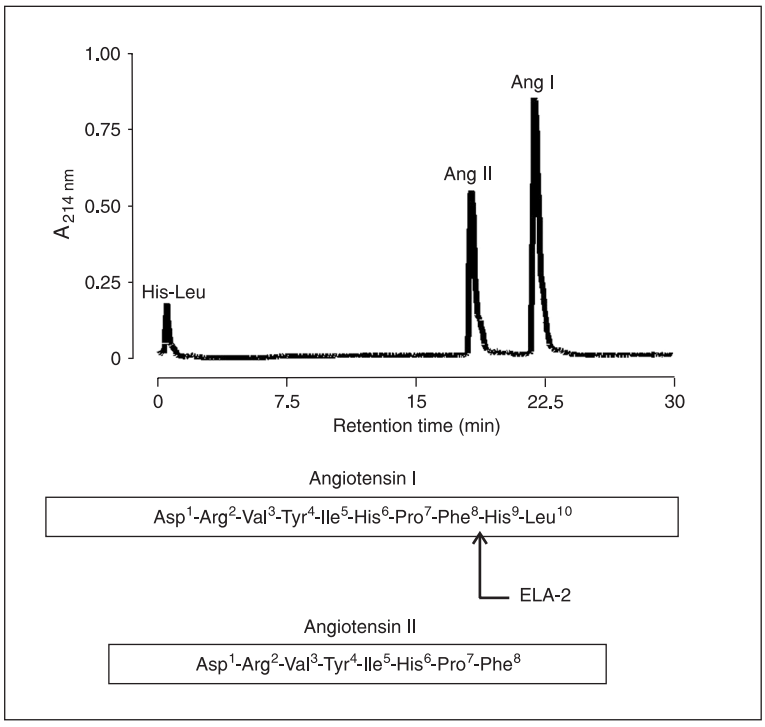

Figure 2. Hydrolysis of angiotensin I (Ang I) by the action of Ang II-forming enzyme purified from rat. Ang I (10 nmol) was incubated with 30 µg elastase-2 (ELA-2) enzyme for 30 min at 37°C. The products of hydrolysis were separated by reversed-phase HPLC with an acetonitrile gradient.

majority of rodent chymases, such as the rat and mouse, that are mainly Ang II-degrading enzymes (29,49,54). Rat and mouse elastase-2 has only Ang II-forming activity. Its catalytic activity for the conversion of Ang I to Ang II is high (Kcat/km = 42.5 mM/min). The fact that elastase-2 interacts with [D-Pro^{11}-Ala^{12}]-Ang I and is sensitive to CH5450 (35), both regarded as a selective substrate and inhibitor for human chymase, suggests that the formation of Ang II by chymases *in vivo*, particularly in the rat, may have been overestimated in previous studies addressing Ang II-forming alternative pathways. In addition, as described for tonin, elastase-2 can also generate Ang II directly from angiotensinogen (22,34,55).

A functional role for elastase-2, as an alternative Ang II-generating pathway in the rat vasculature, was suggested by our laboratory. By using a conductance vessel (carotid) we demonstrated that ACE and elastase-2 are synergistically involved in the generation of Ang II from Ang I in physiological conditions (39). We recently demonstrated that chronic treatment of normotensive and spontaneously hypertensive rats with enalapril induces greater participation of serine proteases in the formation of Ang II from Ang I. This chymostatin-sensitive Ang II generation is markedly increased in the carotid arteries from hypertensive rats and the analysis of elastase-2 mRNA expression showed an increased elastase-2 message in carotid arteries of hypertensive rats. It is interesting to note that increased expression of elastase-2 associated with chronic ACE inhibition is not restricted to the carotid artery, since similar results were observed in hearts of hypertensive rats, indicating that increased expression of elastase-2 upon ACE inhibition may be a widespread phenomenon (56,57). Our recent discovery of elastase-2 as an alternative Ang II-generating pathway in the vasculature indicates that this enzyme may be important in diseases such as arterial hypertension.

The current awareness of the functional complexity of the multifaceted, multicomponent RAS has been reinforced by the identification of different proteases that can process both Ang I and Ang II to generate active metabolites, which can trigger opposing biological responses and might play peculiar regulatory roles in the local RAS. The existence of other enzymes besides ACE generating Ang II, the so-called alternative pathways of Ang II generation, such as human chymase and rat elastase-2, suggests that these proteases may have a role in physiopathological conditions and may underlie the cardiovascular effects of ACE inhibitors.

Acknowledgments

Research supported by FAPESP and CNPq.

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