Although the use of angiotensin converting enzyme inhibitors (ACE-Is) in clinical practice brought the great chance to recognize the RAS role in the physiology and pathology, there are still many questions which we cannot answer. This article reviews actually known pathways of angiotensin II (Ang II) and other peptides of renin-angiotensin system (RAS) production and their physiological significance. The various carboxy- and aminopeptidases generate a range of peptides, like Ang II, Ang III, Ang IV, Ang-(1-7) and Ang-(1-9) possessing their own and known biological activity. In this issue especially the alternative pathways of Ang II synthesis involving enzymes other than angiotensin-converting enzyme (ACE) are discussed. We present many evidences for the significance of a new pathway of Ang II production. It has been clearly shown that Ang I may be converted to Ang-(1-9) by angiotensin-converting enzyme-related carboxypeptidase (ACE-2) and then into Ang II in some tissues, but the enzymes responsible for this process are unknown till now. Although there are many data proving the existence of alternative pathways of Ang II production, we can still block only ACE and angiotensin receptor 1 (AT₁) in clinical practice. It seems that a lot needs to be done before we can wildly complexively control RAS and treat more effectively cardiovascular disorders such as hypertension or heart failure.

Key words: Angiotensin converting enzyme, angiotensin converting enzyme inhibitors, angiotensin, carboxipeptidase, aminopeptidase.

INTRODUCTION

The renin-angiotensin system (RAS) is highly complicated hormonal system controlling cardiovascular system, kidney and adrenal glands, thus crucial for hydro-electrolyte balance and blood pressure regulation. Apparently, RAS is not only endocrine, but also auto- and paracrine system. The final effect of RAS activation is complex and based, on the one hand, on the biological activity of angiotensin II (Ang II), and on the other hand on the activities of other products of Ang I metabolism, exerting often opposite to Ang II action.

In the last few years, the RAS has been newly recognized and its importance is greater than we even thought. Nowadays it is known that there are two RAS systems: plasma-localized, regulating urgent cardiovascular system function and tissue-localized, regulating long-term changes. Furthermore, new enzymes have been described and our knowledge about pathways of angiotensins production expanded. The recently accepted metabolism of Ang I in plasma and tissues are described below and presented on Figure 1.

THE PLASMA PATHWAYS OF ANG I METABOLISM AND THE BIOLOGICAL FUNCTION OF ITS MAIN PRODUCTS.

Plasma RAS it thought to be endocrine system. Released from juxtaglomerular apparatus renin cleaves of Leu-Val peptide bond at N-terminus of angiotensinogen, generating decapptide - Ang I. At a next step the dipeptide (His-Leu) is cleaved form Ang I at C-terminus to generate Ang II by angiotensin converting enzyme (ACE). ACE is a dipeptidyl carboxypeptidase glycoprotein weighting 90-100 kD or 140-160 kD, dependently to localization (1). It is classified as a M2-family metalloprotease containing one zinc ion in its structure. Moreover, ACE is identical to kininase II, thus it degradates bradykinin, which was previously widely described (2). Additionally ACE is potent to convert Ang-(1-9) into Ang-(1-7) (3). Furthermore, at the N-terminus, Ang II is cleaved by aminopeptidase A (APA) to form Ang III, which is depleted of the last N-terminal aminoacid by aminopeptidase N (APN) generating Ang IV. Ang IV, in turn is degradated into small fragments. Endopeptidases may also cut off Asp from the N-terminus of Ang I forming Des-Asp^{1}-Ang I (DAA-I), which, in turn is cleaved by ACE directly into Ang III (4).

Angiotensin II [Ang-(1-8)]

Ang II is the best described peptide of RAS. Its properties in physiology and pathology of cardiovascular system had been widely discussed in previous review articles (4-6). Shortly, Ang II increases activity of sympathetic nervous system, acts as a vasoconstrictor, increases aldosterone release and sodium retention (4). Additionally, Ang II stimulates free radical production,
plasminogen activator inhibitor - 1 (PAI-1) release, tissue factor (TF) and adhesion molecules (VCAM-1) expression. Moreover, in blood vessels it stimulates smooth muscle cells proliferation and leukocyte adhesion. What is important, Ang II inhibits nitric oxide synthase (NOS), thus diminishing all beneficial effects of nitric oxide (NO) (6). We have also found that Ang II enhances venous and arterial thrombosis development in rats (7, 8). Recently it has been shown that Ang II in the presence of ACE-I and AT1 receptor blocker (ARB) increases duodenal HCO3− secretion via a common pathway, involving bradykinin, NO and prostaglandis (9).

Angiotensin III [Ang-(2-8)]

Similarly to Ang II, Ang III is also a vasoconstricting factor. After intravenous infusion into healthy volunteers and hypertensive patients it increases blood pressure about 20 mmHg (10) and augments aldosterone concentration (11). Ang III had 25% of the pressor potency of Ang II when tested using acute intravenous administration into rat (12). It is also postulated that Ang III is responsible for central regulation of blood pressure. Indeed, in rats injection into lateral cerebral ventricles of the selective APA inhibitor EC33 [(S)-3-amino-4-mercaptobutyl sulfonic acid] blocked the pressor response of exogenous Ang II (13). Similarly to Ang II, Ang III concentration increases during development of renal hypertension in rat. Moreover, Ang III may increase expression of growth factors, like TGF-β1 and proteins of extracellular matrix, like fibronectin (14). Furthermore, in vitro Ang III is a chemoattractant factor for polymorphonuclear leukocytes (PMN's) (15). All these activities makes this peptide less potent, but similar to Ang II.

Angiotensin IV [Ang-(3-8)]

Some authors report that Ang IV is a vasorelaxative agent and this effect is contributed to activation of endothelial NOS (16). Nevertheless intravenous infusion of this peptide does not affect mean blood pressure (17). On the other hand Ang IV, like Ang II, seems to be a proliferative agent and Ang IV receptor - (AT4 receptor) is involved in this effect (18). Moreover it has been proved that Ang IV stimulates the activity of tyrosine kinases (PTK) in experimental rat pituitary tumor and in normal rat anterior pituitary tissue (19).

Angiotensin-(1-9)

Ang-(1-9) is relatively poorly known peptide. Physiological concentration of Ang-(1-9) in human and rat plasma is very low (20), but in kidney it reaches about 50% of Ang I concentration (21). It is strong, competitive inhibitor of ACE (at multiple-fold lesser concentration than Ang I) (22) and like Ang-(1-7), due to enhanced bradykinin action on its B2 receptor, increases nitric oxide and
arachidonic acid release. Moreover, the action of Ang-(1-9), is significantly stronger when compared to the effect of Ang-(1-7) (23).

Because Ang-(1-9) is probably the main product of Ang I metabolism in platelets (24), its involvement in the regulation of platelet function is possible. Our preliminary experiments showed that Ang-(1-9) inhibits in vitro collagen-induced platelet aggregation in rat (25).

Angiotensin-(1-7)

Ang-(1-7) is an active peptide of RAS. It counteracts vasoconstriction by releasing nitric oxide and prostacyclin (26). Moreover, it opposites Ang II mitogenic, arrythmogenic and procoagulant activities (4). Enhancing natriuresis and diuresis it inhibits water and sodium retention caused by Ang II. Recently it has been shown that vasodilatative and diuretic activities of Ang-(1-7) are mediated via Mas, G- coupled protein receptor (27). Furthermore, some activities of Ang-(1-7) are blocked by AT$_1$ and AT$_2$ receptors antagonists (26). On the other hand, Ang-(1-7) independently to Mas-receptor increases bradykinin activity and antagonizes hypertrophic action of Ang II (28). In 2002 non-peptide antagonists of Ang-(1-7) receptor have been described (29).

TISSUE RAS

Local synthesis of Ang peptides begins when angiotensinogen penetrates from plasma into a tissue. It is known that angiotensinogen is not synthesized in situ, thus has to be produced in liver and distributed with plasma (30, 31). At the next step angiotensinogen is enzimatically cleaved by renin - free or bound to cell membrane. According to actual data, renin and prorenin are not synthesized outside of juxtaglomerular apparatus, but they are bounded and internalized by their own peripheral tissues renin receptors (32, 33). On the other hand, another observations indicated alternative, independent to renin, pathways of Ang II synthesis from angiotensinogen. In in vitro experiments it was shown that Ang II may be produced directly from angiotensinogen by tissue-type plasminogen activator (t-PA), cathepsin G, tonin, trypsin and chymotrypsin (34-36). Until now it has not been established which of these pathways is significant in vivo. First, some of postulated alternative enzymes, for example catephsin D, produce Ang II under non-physiological pH values (37). Furthermore, the total lack of Ang I and Ang II in animal and human plasma and tissues after bilateral nephrectomy questions the existance of non-renin pathways of angiotensins synthesis (38). In tissues, like in plasma, Ang I is converted into Ang II mostly by bound to cell membrane ACE. Experiments conducted on Langendorff hearts showed that newly generated Ang II immediately bounds to the angiotensin receptors or it is internalized and stored inside the cell (39).
In tissues Ang II is also converted by angiotensin-converting enzyme-related carboxypeptidase (ACE-2) (40) or by poorly identified carboxypeptidase P to Ang-(1-7), which reaches very high concentrations when compared to plasma. Another substrates for Ang-(1-7) production are Ang I and Ang-(1-9). The Ang-(1-7) peptide is synthesized by specific endopeptidases cutting of three amino acids: Phe, His and Leu at C-terminus of Ang I. Additionally, in the endothelial cells of human, porcine and bovine aorta and in human umbilical cord vein, the main enzymes converting Ang I and Ang-(1-7) are: neprylizin and pyrrolic endopeptidase and in smooth muscle cells of normotensive or spontaneously hypertensive rats - thiomethyl oligopeptidase (41). As mentioned before, Ang-(1-7) is synthesized directly from Ang-(1-9) by ACE cutting of C-terminal Phe and His from Ang-(1-9) (42). In brain Ang II is degraded not only to Ang-(1-7) but also, like in plasma, to Ang III, Ang IV and small fragments (Fig. 1). Because of both renin and angiotensinogen do not penetrate the blood-brain barrier, it seems that all RAS elements are synthesized locally in the central nervous system (CNS). Indeed, the main amounts of angiotensinogen are synthesized by astrocytes. Probably the enzyme responsible for Ang I synthesis in CNS is cathepsin D. Furthermore, in the brain the presence of aminopeptidases A and N synthesizing Ang III an Ang IV is also well documented (43). But yet exact localization of angiotensin peptides synthesis in CNS remains unknown.
ALTERNATIVE PATHWAYS OF ANG II PRODUCTION.

It is well established, that are many findings indicating that ACE-Is totally inhibit Ang II generation both in plasma and tissues, proving that ACE play crucial role for Ang II synthesis (44). We have recently shown that there are some pharmacological differences among various ACE-Is (45, 46). It should not be excluded than, that ACE-Is may be non-selective. Moreover, in many patients treated with ACE-Is blood pressure does not decrease whereas aldosterone concentration increases (47). Interestingly, in normotensive and hypertensive rats after 14-day therapy with ceranapril or lisinopril plasma concentration of Ang II grows multiple-fold. The authors of this observation suggest that the activity of unknown enzymes metabolizing Ang I increase just as a result of ACE-Is presence (48). But still the main problem remains unsolved: why, despite of ACE blockade, Ang II is generated in plasma? Many authors proved an existence of alternative, independent to ACE, pathways of Ang II synthesis form Ang I under in vitro conditions (49-54).

**Chymostatin-sensitive Ang II Generating Enzyme (CAGE) - dependent pathway of Ang II production**

Experiments with isolated aortas showed, chymostatin-sensitive enzyme, generating Ang II from Ang I - CAGE (49). However, its role in physiology is still unclear.

**Chymase - dependent pathway of Ang II production**

In 1991 another enzyme was isolated and clonned - the heart chymase, which was being suggested to be responsible for Ang II synthesis in the heart (50). Moreover, kinetic investigations showed, that chymase produces at least 90% of Ang II in heart (51). However, it seems that this enzyme is crucial only under pathological conditions, eg. in ischemic heart, because it is accumulated in inflammation cells - mastocytes. Besides the heart chymase has been discovered nearly 15 years ago, effectiveness of chymase inhibitors in therapy of cardiovascular system was not yet confirmed.

**ACE-2 - dependent pathway of Ang II production**

In 2000 a new enzyme cleaving Ang I into Ang-(1-9) was identified (52) (Fig.1). It is called angiotensin-converting enzyme-related carboxypeptidase (ACE-2). Like ACE, it is a zinc metaloprotease weighting about 120 kD. First ACE-2 was identified from 5' sequencing of a human heart failure ventricle cDNA library. Unlike to ACE, ACE-2 in vitro cuts off a single aminoacid from Ang I or Ang II, forming Ang-(1-9) and Ang-(1-7), correspondingly (53). Nevertheless, catalytic activity of ACE-2 vs Ang II is even 400-fold higher in comparison to that vs Ang I (54). Moreover, it is not inhibited by ACE-Is and it
is involved in synthesis of other active peptides like apelline-13 or dynorphine A. ACE-2 is present in macrophages, endothelial and smooth muscle cells. ACE-2 gene expression is described in cardiovascular system (53), in renal cortex and medulla (53, 55), some tissues of gastrointestinal tract (55) and in testis (56). Interestingly, changes in ACE-2 expression were observed in various physiological and pathological conditions, for example during pregnancy, in hypertension, in heart and renal failure and in diabetic patients (53-59). In glomeruli of diabetic mice the level of ACE-2 grows, when ACE decreases in the same time, suggesting nephroprotective role of ACE-2 in early stages of diabetes mellitus (57). Furthermore, it is proved that after myocardial infarction, both in rat and human, ACE-2 expression in various tissues increases, indicating the role of ACE-2 dependent pathways counteracting of negative effects of RAS activation in states after heart dysfunction (58). In turn, in rats with three various models of hypertension, mRNA for ACE-2 decreases (59). Thus, it is possible, that all beneficial ACE-2 dependent effects may be a result of biological action of products of this enzyme: Ang-(1-9) and Ang-(1-7).

After describing of ACE-2 the biological significance of a new alternative pathway of Ang II production became more probable. First, it has been clearly shown that Ang I may be converted to Ang-(1-9) by ACE-2 (52, 53). Second, many interesting previous findings indicate that Ang-(1-9) may be converted into Ang II in some tissues, but the enzymes responsible for this process are unknown till now. Drummer et al. proved that homogenates of rat kidney, and in a lesser extent lung, converts Ang-(1-9) to Ang II, due to ACE-independent aminopeptidase and N-like carboxypeptidase (60) (Fig. 1). Theoretically (kinetic investigations in vitro) it is known that Ang-(1-9) is metabolized by ACE to Ang-(1-7), and further to Ang-(1-5) and Ang-(1-4) (52). Nevertheless, Drummer et al (60) showed that in the kidney the main product (71%) of Ang-(1-9) conversion is Ang II, accompanied by small amounts of Ang III and Ang-(2-9). Unfortunately, the use of poorly specific inhibitor of the sequent conversion in kidney (cobalt, EDTA, iodoacetic acid) did not allow to clearly identify the enzyme responsible for this reaction (60). Furthermore, in 2005 Singh et al. confirmed that the pathway: Ang I - Ang-(1-9) - Ang II really exists in glomeruli of streptozotocin-induced diabetes mellitus rats (61). Moreover, in human heart tissue the main products of Ang I degradation are both Ang-(1-9) and Ang II generated by heart chymase, ACE and poorly identified carboxypeptidase A (22). However, it is still not established whether Ang-(1-9) may occur in plasma under physiological conditions. Many investigators failed to measure Ang-(1-9) level in plasma, but some found even higher than Ang II concentration of Ang-(1-9) (21, 62). Another riddle is the source of Ang-(1-9) found in plasma. Is it generated on the endothelial cells surface (similarly to ACE action), or rather Ang-(1-9) is produced and secreted by platelets into the blood? Snyder et al. showed that the main metabolite of Ang I in platelet is not Ang II but Ang-(1-9), which
simultaneously inhibits ACE (24), but did not examine whether Ang II can be produced from Ang-(1-9).

**CONCLUSION**

Despite of over 100 years passed after Tigerstedt and Bergman discovered RAS, our knowledge about this system remains incomplete. We know there is a highly complex tissue RAS, involving multiple ways of Ang II production. Apart that, Ang I is the source of other active peptides, like Ang-(1-7) or Ang-(1-9). Thus, it seems that the result of the activation of RAS in tissues is the joint effect of several peptides. Their production is determined by the activity of various enzymes, well-known, like ACE, chymase or CAGE or newly described, like ACE-2. Although there are many evidence proving the existence of alternative pathways of Ang II generation, we still block only ACE and AT\(_1\) receptor in clinical practice. It seems that a lot needs to be done before we can wisely control RAS and treat more effectively cardiovascular disorders such as hypertension or heart failure.

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**REFERENCES**


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Author’s address: Prof. dr hab. Wlodzimierz Buczko, Head of Department of Pharmacodynamics, Medical University of Białystok, 2C Mickiewicza Str., 15-089 Białystok, tel/fax: (48-85) 748 56 01; e-mail: pharmdyn@amb.edu.pl