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Current Medicinal Chemistry, 2013, *20*, ????-???? 1

The Renin-Angiotensin System: New Insight into Old Therapies

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Abstract: Although the renin-angiotensin system (RAS) is already an old acquaintance, there are often exciting discoveries that improve our knowledge of it and open new therapeutic possibilities. Moreover, well-established drugs, such as angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), or beta-blockers, show that their mechanism of action may be the result of parallel pathways other than the ones initially established. A detailed analysis of the RAS can be carried out in part through the study of the enzymes, named angiotensinases, involved in its cascade, whose activity is a reflection of the functionality of their peptide substrates. The study of these enzymes offers the possibility of controlling the effects of angiotensins through various pharmacological manipulations. For example, angiotensinase inhibitors or activators are being used or have been proposed as antihypertensive agents. They have also been suggested as analgesic and antidepressant drugs or targets for drug development against different pathologies such as Alzheimer's disease, epilepsy or ischemia. On the other hand, the analysis of brain asymmetry has revealed surprising results about the laterality of central and peripheral components of the RAS. Such studies indicate that the *neurovisceral integration*, already proposed by Claude Bernard (1867) should also be analyzed from a bilateral perspective. In this review, the RAS and the role of various angiotensinases implicated in the cascade are revisited. Therapeutic strategies involving some components of the RAS with an unusual vision resulting from a bilateral perspective added to their study are discussed.

Keywords: Renin-angiotensin system, angiotensinases, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers, brain asymmetry.

THE RAS REVISITED

In 1898, Tigerstedt and Bergman [1] extracted a substance from the rabbit renal cortex that exhibited pressor effects after intravenous administration. They named it renin because of its tissue origin. Although these authors probably did not realize the importance of their discovery, it constituted a key point, a before and after, in our understanding of the hormonal regulation of blood pressure (BP) [2]. The renin-angiotensin system (RAS) is an excellent example of the relationship between structure and function in biology. Small changes in structure, i.e. deletion of one or two amino acids from an angiotensin (Ang) active peptide precursor, result in significant changes in function. In that process, proteolytic enzymes such as the exopeptidases play a major role.

In the classic view of the systemic RAS, the appropriate stimuli, such as decreases in BP, reduction in plasma sodium concentration and plasma volume or sympathetic stimulation, lead to the release of the enzyme renin from the juxtaglomerular apparatus of the kidney. Renin is the specific limiting factor that activates the enzymatic cascade. This enzyme is an endopeptidase that cleaves the substrate precursor angiotensinogen (AOGEN) to produce angiotensin I (Ang I or Ang 1-10). Alternatively, renin may derive from its inactive precursor prorenin (PRR) through proteolytic activation by the enzymes proconvertase 1 or cathepsin B, which remove the prosegment [3]. Inactive PRR may also undergo a non-proteolytic activation under conditions such as low pH or low temperature leading to an unfolding of the prosegment due to conformational change, or by direct binding to the prorenin receptor (PRR-R). In both cases, PRR became enzymatically active and may hydrolyze AOGEN to produce Ang I. Independently of the effects produced by the various angiotensin peptides, the binding of PRR mostly, but also of renin to the PRR-R, may induce increased contractility, cardiac hypertrophy and fibrosis, glomerulosclerosis and apoptosis [3, 4] Fig. (**1**).

Ang I, produced by the action of renin or by activated prorenin, is metabolized by various angiotensinases (AG) i.e. enzymes that act on angiotensin peptides [5], to produce the diverse active peptides of the system. In the standard way, angiotensin converting enzyme (ACE), a peptidyl-dipeptidase or dipeptidyl carboxypeptidase, ubiquitously expressed with highest levels in lung epithelium, kidney, heart and gastrointestinal system acts on Ang I [6]. This enzyme splits the carboxy-terminal His^{9}-Leu^{10} dipeptide producing the most active peptide of the system: Ang II (Ang 1-8) which is further metabolized to Ang III (Ang 2-8) by glu-

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0929-8673/13 $58.00+.00

tamyl-aminopeptidase or aminopeptidase A (GluAP, AP A), an enzyme particularly abundant in kidney [7], which deletes the amino-terminal Asp^1 residue. However, Ang I may be the source of other pathways depending on the AG that act on it; i.e. aspartyl-aminopeptidase (AspAP), an enzyme different of GluAP [8, 9] detaches the amino-terminal Asp^1 amino acid, producing Ang 2-10, which could be further converted to Ang III by action of ACE. Alternatively, Ang I may be converted to Ang 1-7 by neutral endopeptidase (NEP), splitting the tripeptide carboxi-terminal Phe^8-His^9-Leu^{10}. Ang 1-7 may then be metabolized to Ang 2-7 by AP A, removing Asp^1 or be converted to Ang 1-5 by ACE, breaking the carboxy terminal dipeptide His^6-Pro^7. Ang 1-7 may be also produced from Ang II (1-8) by action of carboxypeptidase P (Carb-P) or by the ACE homologous ACE_2, an enzyme that is not inhibited by ACEI and that acts as a carboxypeptidase by removing the carboxy-terminal amino acid [10], in this case Phe^8. Ang I (Ang 1-10) may be also metabolized to Ang 1-9 by action of ACE_2, which removes the C-terminal Leu^{10} residue. Ang 1-9 could be then converted to Ang 1-7 by ACE splitting the dipeptide C-terminal Phe^8-His^9. Ang III (Ang 2-8) the main metabolite of Ang II, could be converted to Ang IV (Ang 3-8) through the action of arginyl-aminopeptidase (AP B) or alanyl-aminopeptidase (AP M, AP N) that cleaves its amino-terminal Arg^2 residue. Depending on the action of Carb-P or AP M, Ang IV (Ang 3-8) will be respectively converted to Ang 3-7 (by cutting Phe^8) or Ang 4-8 (by separating Val^3). Finally, Ang 4-8 could be converted to Ang 5-8 removing Tyr^4 by the adipocyte-derived leucine aminopeptidase (A-LAP) [11, 12] Fig. (**2**).

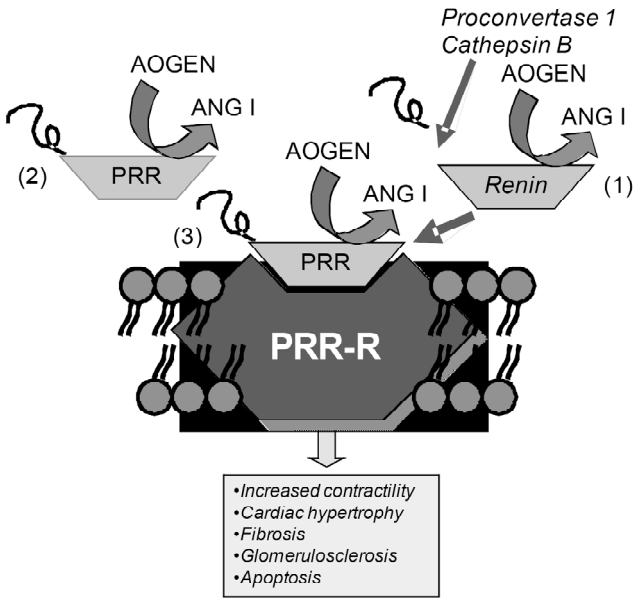

Fig. (1). Schematic representation of proteolytic and non-proteolytic activation of prorenin. (**1**) Renin may derivate from its inactive precursor prorenin through proteolytic activation by the enzymes proconvertase 1 or cathepsin B, which remove the prosegment. (**2**) Inactive prorenin may also undergo a non-proteolytic activation under conditions such as low pH or low temperature leading to an unfolding of the prosegment due to conformational change, or (**3**) by direct binding to the prorenin receptor. In both cases, prorenin became enzymatically active and may hydrolyze angiotensinogen to produce angiotensin I (see text for abbreviations).

In this complicated network of various metabolic pathways, we recognize the importance of AG controlling which of those pathways will be activated or inhibited and therefore leading to the predominance of one or another active Ang peptide and consequently, activating one or another specific function. This is particularly interesting if we consider the role of the brain RAS in hypertension. In brain, the metabolism of Ang II to Ang III is a key step since Ang III has been proposed to be the most active Ang metabolite exerting a tonic stimulatory effect on BP [13]. The enzyme that generates Ang III from Ang II is GluAP (AP A). Therefore, the development of inhibitors of this enzyme acting at a central level has been a new target for the treatment of hypertension [13].

NEW ANGIOTENSIN ACTIVE PEPTIDES

If the major role of the RAS in the regulation of BP, haemodynamic or hydroelectrolytic balance is well recognized, this system is also involved in multiple other functions including the cognitive ones [12, 14, 15].

Until recently, Ang II was considered as the major peptide of the RAS controlling the increase of BP. It also contributes to the progressive dysfunction of end organs such as blood vessels, kidneys, liver or heart, these deleterious effects being attenuated by the use of ACEI [16-19]. Today this concept is changing and other active Ang peptides such as Ang III, Ang IV, Ang 1-9, Ang 1-7 or Ang 2-10 exhibit not only functions that may counteract the ones of Ang II but also others which are not necessarily related to the cardiovascular system [11, 12, 20].

Angiotensin III

The Ang III derivative, a less potent vasoconstrictor than Ang II [21], stimulates aldosterone secretion [22], partially through its binding to the AT_2 receptor [23], has clear dipsogenic activities and is a neuronal stimulus [24]. It binds to both AT_1 and AT_2 receptors with similar affinity [25, 26]. Ang III is also involved in renal damage [27] and owns antinociceptive activity through its action on the AT_1 and AT_2 receptors [28]. Finally, it is proposed that Ang III is the key active form of the central angiotensins, exerting tonic stimulatory control over blood pressure [13, 29].

Angiotensin IV

Ang IV appears to play a role in regulating local blood flows [30] in organs that include the brain [31]. This peptide may have memory-potentiating effects via actions on glucose transport or neuropeptide processing [32]. It has anxiolytic properties promoting the increase of oxytocin in the rat amygdala [33]. The group of Ruiz-Ortega [34] suggested that Ang IV might contribute to inflammatory events in cardiovascular diseases via activation of the nuclear transcription factor-kappa B pathway and the regulation of proinflammatory genes. Ang IV may have a protective effect on Ang II-induced cardiac injury and dysfunction [35]. Finally, it has been reported that Ang-IV improved glucose tolerance, insulin signaling, and para-inflammatory processes linked to hyperglycemia [36].

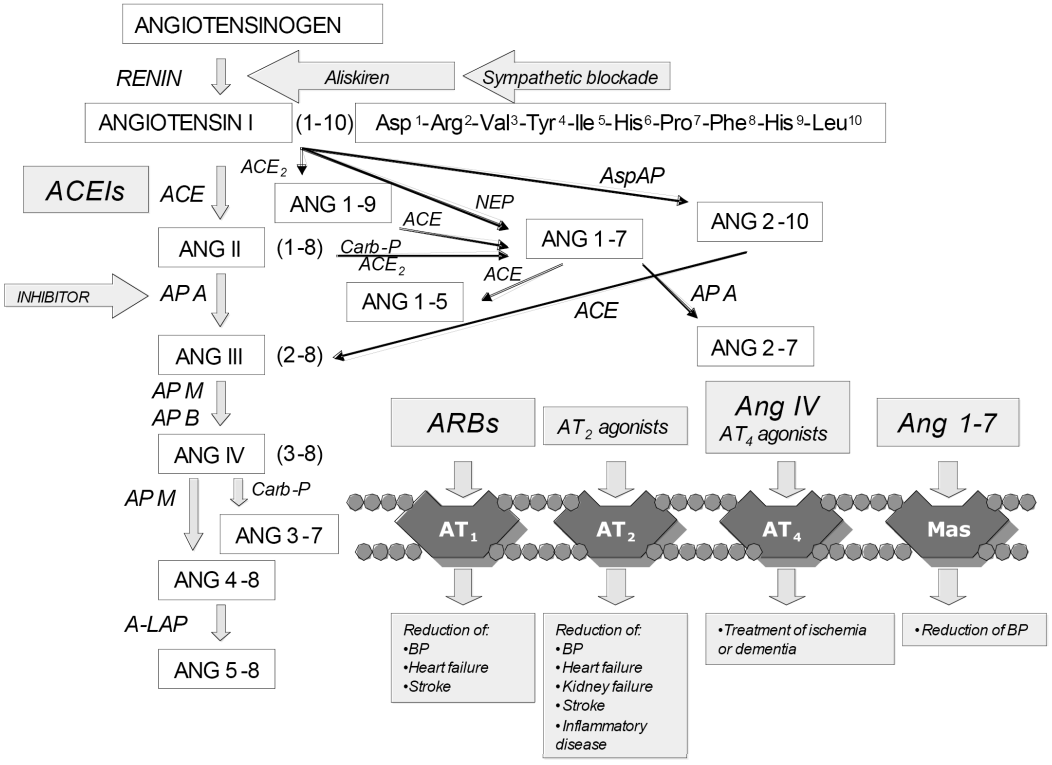

Fig. (2). Partial representation of the renin-angiotensin system. The strategies of blockade of the hypertensive actions of the system, such as the inhibition of renin with Aliskiren, the inhibition of angiotensin-converting enzyme, the inhibition of aminopeptidase A (in brain), the blockade of AT1 receptors or the sympathetic blockade through the use of beta-blockers are indicated (see text for abbreviations).

Angiotensin 1-7

One of the most studied Ang peptides is Ang 1-7. This peptide has been demonstrated to be a hypotensive agent that counteracts Ang II through its binding to the *mas*-receptor. This peptide antagonizes the actions of Ang II increasing the vasodilatory effects of bradykinin, stimulating nitric oxide (NO) and prostaglandin release and exerting a diuretic and natriuretic effect [37]. Microinjection of Ang 1-7 into brainstem nuclei of the rat promotes hypotension [38, 39]. It has been reported that Ang 1-7 is gastroprotective [40] and posses a protective role in the acute respiratory distress syndrome [41]. The use of ACEI increases the availability of Ang 1-7 which may significantly contribute to the hypotensive and beneficial effects of ACEI. ARB also increases Ang 1-7 through a rise in the expression of ACE_2 [34]. Based on the beneficial effects of Ang 1-7, potential activators of ACE_2 have been reported that decreased BP, improved cardiac function and reversed myocardial and perivascular fibrosis in spontaneously hypertensive rats (SHR) [39, 42].

Angiotensin 1-9

Although formation of Ang 1-9 has been considered only as an intermediate step in the formation of Ang 1-7, some authors suggest that this is an active Ang peptide by itself which may also counterbalance Ang II probably by its binding to the AT_2 receptor [43].

Angiotensin 2-10

Peripheral and central studies have suggested the involvement of Ang 2-10 in hypertension. In the kidney, it has been observed that Ang 2-10 counteracts the action of Ang II [44]. It was also proposed that Ang 2-10 possesses protective vascular actions in kidney and mesenteric beds attenuating the pressor action of angiotensin III in both normotensive and hypertensive rats [45]. However, although Ang 2-10 may counterbalance the actions of Ang II or Ang III in peripheral tissues, it is not demonstrated that brain Ang 2-10 would have similar effects. Sim and Radhakrishnan however have reported that intracerebroventricularly administered des-Asp-angiotensin I (Ang 2-10) attenuated the pressor effect of Ang II and Ang III in SHR and Wistar Kyoto rats [46]. Unpublished results by our group suggested that Ang 2-10 might counterbalance Ang III in hypothalamus under propranolol treatment. In addition, as mentioned for Ang IV, Ang 2-10 also improve glucose tolerance and insulin signaling in diet-induced hyperglycemic mice acting through its binding to the AT_1 receptor [47].

ANGIOTENSIN RECEPTORS

Classically, the effects of Ang II were resulting to its binding to one unique receptor, the AT_1. However, the existence of another subtype of Ang receptor that bound Ang II was reported more than twenty years ago [48]. Later, a new receptor subtype, that mainly bound Ang IV, was identified by Harding *et al.* in 1992 [49]. Finally, it was reported that

the Mas proto-oncogene was a receptor that bound specifically Ang 1-7 [50]. Therefore, at present, there are four characterized receptor subtypes that bind Ang II and the other active Ang metabolites with various affinities Fig. (**2**). The AT_1 receptor binds Ang II with the highest specificity but it also binds Ang III; the AT_2 receptor binds mainly Ang III and Ang II but also Ang 1-9 [20, 51, 52]; the AT_4 receptor binds specifically Ang IV and Mas-receptor binds uniquely Ang 1-7. AT_1 receptor activation is related to hypertension, heart failure, atherosclerosis, left ventricular cardiac hypertrophy, diabetic nephropathy, retinopathy or stroke. Therefore, its blockade with ARB may reduce such deleterious effects. The recent development of a selective non-peptidic receptor agonist for AT_2 has revealed functions such as antiproliferative, (neuro)-regenerative or anti-inflammatory, all of them counterbalancing the adverse effects of AT_1 activation, supporting the Yin and Yang hypothesis [53].

In contrast to Ang III, the affinity of Ang IV for the AT_1 and AT_2 receptors is low [25], but it shows a high affinity for the AT_4 receptor, leading in part to the regulation of local blood flows [30, 31]. In contrast to the concept that AT_4 is identified with the type I tyrosine kinase receptor, c-Met [12], it has been also reported that this receptor is identical to the insulin-regulated aminopeptidase (IRAP) [54]. Following this observation, it has been suggested that activation of the AT_4 receptor may play a role in the improvement of cognitive functions. Indeed, it was proposed that the binding of Ang IV to its receptor, AT_4 (IRAP), results in the inhibition of the receptor's metabolic activity, reducing the catabolism of its substrates (vasopressin, oxytocin) and consequently increasing their availability and extending their action (55). Ang IV could therefore regulate glucose uptake in modulating IRAP, which is indeed co-localized with the glucose transporter GLUT4. In the presence of insulin, IRAP and GLUT4 are expressed in the plasma membrane, where GLUT4 induces glucose uptake. It was suggested that the inhibition of IRAP, following binding of Ang IV, could increase glucose uptake in neurons leading to an improvement of cognitive processes [55-58]. Therefore, if activation of AT_4 receptor improves brain blood flow and cognitive functions, the development of agonists of AT_4 or elements that increase the availability or prolong the half life of Ang IV, may be useful in the treatment of ischemia or dementia, including Alzheimer's disease [59]. In addition to its location in brain, the AT_4 receptor has a broad distribution in tissues such as the adrenal gland, kidney, lung and heart. In the kidney, Ang IV increases renal cortical blood flow and decreases Na(+) transport in isolated renal proximal tubules [32].

Finally, it has been reported that the Mas proto-oncogene is a receptor for Ang 1-7 [50]. The Mas receptor through its activation with Ang 1-7 posses antiproliferative and antiarrhythmic effects, produces vasodilation via bradykinin and NO-release, stimulates renal sodium excretion and the sympathetic nervous system function [37].

NEW PERSPECTIVES FOR AN OLD SYSTEM

Several pharmacological strategies are currently used to counteract the deleterious actions of Ang II: direct blockade of its actions either by inhibiting its formation with renin or ACEI or blocking the receptor with ARB, or indirectly using sympathetic antagonists such as beta-blockers [60]. Other pharmacological strategies Fig. (**4**) are also considered such as the search for inhibitors of specific enzymes of the system that reduce the hypertensive Ang peptides, such as Ang II at the periphery or Ang III at the central level. As an example, in a series of elegant studies, the group of Llorens-Cortes has developed a new class of centrally acting antihypertensive agent, such as the potent orally active AP A inhibitor RB150 [13]. In addition, new efforts are being made to search for activators of the enzymes responsible for the generation of Ang peptides that could counteract the deleterious actions of Ang II such as Ang 1-7, Ang 1-9 or Ang 2-10. Therefore, since ACE_2 is responsible for the formation of Ang 1-7 and Ang 1-9 (see Fig. **2**), both counteracting the adverse effects of Ang II, the development of ACE_2 activators are potential therapeutic tool.

Hernández-Prada *et al.* [42] have identified two compounds, named xanthenone and resorcinolnaphthalein that enhanced ACE_2 activity in a dose-dependent manner Fig. (**4**). *In vivo* administration of xanthenone resulted in a decrease in BP in normotensive and hypertensive rats. In addition, the authors observed an improvement in cardiac function as well as a reversal of myocardial, perivascular and renal fibrosis in the SHR. It has been proposed that the pharmacological activation of ACE_2 promotes an increased Ang 1–7 production with concomitant degradation of Ang II explaining its beneficial effects [39]. Alternatively, this outcome might also be due to the activation of Ang 1-9 formation from Ang I (see Fig. **2**) and.modification of other pathways of the RAS under the activation of ACE_2 cannot be ruled out.

In addition, several data suggests that Ang 2-10 may have beneficial effects, not only counteracting the adverse actions of Ang II at the periphery and of Ang III in the brain, but also in diabetes mellitus. Thus, the pharmacological search of activators of AspAP, the enzyme that metabolizes Ang I to Ang 2-10, could also be of interest [44-47]. Strategies for such a search, already initiated in our laboratory, include the analysis of AspAP activity in the presence of small molecules, mainly heterocyclic as pyrimidine and its fused derivatives. This could allow us to perform in-silico screening with small molecules available in large libraries such as the National Cancer Institute Developmental Therapeutics Program to identify potential molecules enhancing AspAP activity [61].

OTHER ACTIONS OF ANTIHYPERTENSIVE AGENTS

Except if one blocks the RAS cascade at its early step by inhibiting renin with compounds such as Aliskiren [62], the alteration of other steps using ACEI or ARB may affect the rest of system. Furthermore, the use of other antihypertensive agents, such as beta-blockers, may also influence the RAS, promoting or inhibiting the formation of specific Ang peptides. These possibilities however have not been systematically considered. Currently, there are increasing evidences that the beneficial effects of these drugs are not only the consequence of their blockade of Ang II or sympathetic actions but also the result of the increased formation of other Ang peptides that counteract the detrimental actions of Ang II.

Beyond the reduction of Ang II formation, ACEI exhibit other pleiotropic actions that might also influence their pharmacological benefits. For example, it has been observed that the chronic use of captopril in patients with essential hypertension reduces BP not simply because of the inhibition of Ang II formation but since an increased formation of Ang 1-7 and prostacyclin is noticed [63]. The involvement of Ang 1-7 after ACEI and ARB is supported by the fact that blockade of Mas, the Ang 1-7 receptor, reduces their effect [39]. Ocaranza and Jalil [43] have reported an increase in plasma ACE_2 activity in rats after enalapril. In that study however, plasma Ang 1-9 levels increased without changes in Ang 1-7. The authors suggest that enalapril may promote the conversion of Ang I to Ang 1-9 through ACE_2 rather than the formation of Ang 1-7 through NEP.

Alternative mechanisms for ACEI should also be considered, because other pathways than the metabolism of Ang I to Ang 1-9 or to Ang 1-7, may be affected by activation or inhibition of ACE. Recently, it was reported that after captopril treatment, there was an overproduction of Ang 2-10 in the hypothalamus due to an increase in AspAP activity, responsible for the metabolism of Ang I. This Ang 2-10 formation may locally counteract the action of Ang II. In plasma, there was an increment of vasopressinase activity together with a raise of AP M, suggesting an increase in vasopressin (ADH) catabolism and a promotion of Ang IV formation, respectively (see Fig. **2**). Therefore, the reduced availability of ADH concomitant with the higher formation of Ang IV in plasma may lead to the inhibition of vasopressinase activity in the cells of the inner medulla collecting duct, as observed in the Villarejo's study [64]. In addition, it could be speculated that beyond the reduction of BP and its cardioprotective effects, the additional beneficial effects of captopril, such as the improvement of insulin sensitivity [65], energy balance and glucose homeostasis [66], could be partly due to the production of other Ang peptides such as Ang IV [64] involved in the regulation of IRAP activity and therefore in the glucose transmembrane transport through the control of GLUT4 function [47]. Interestingly, IRAP is co-localized with GLUT4 [67-69].

On the other hand, the treatment with captopril has recently revealed surprising results that go beyond the classic effects of this drug on the enzymatic cascade of the RAS [70]. The analysis of brain asymmetry after captopril treatment has revealed unexpected results about the bilateral behavior of some brain AG activities and the connection with their plasmatic activities. These studies have suggested that the "*neurovisceral integration model"*, i.e., the intimate interaction between brain and cardiovascular system, firstly postulated by Claude Bernard in 1867 [71] and extensively elaborated during the last decade by Thayer and Lane [72, 73], should also be analyzed from a bilateral perspective. The aim of Segarra *et al.* [70] was to analyze the peripheral response of plasma AG activities and to try to compare it with the activities of soluble (Sol) or membrane bound (MB) AG of left or right frontal cortex (FC) in control and captopril-treated SHR. Captopril modified the bilateral distribution of AG activities observed in the frontal cortex of control animals. Interestingly, significant correlations between left or right FC (LFC, RFC) and plasma AG were also observed. In control animals, only the AG of RFC correlated significantly with plasma activities: the correlation was negative for Sol but positive for MB activities. In contrast, in the group of captopril-treated rats, only the AG of LFC correlated with plasma AP, again negatively for Sol and positively for MB Fig. (**3**). Additional results obtained in our laboratory also indicate that the bilateral pattern of correlations between FC AG activities and heart ventricle AG observed in control SHR is also modified after captopril treatment [74]. These results possibly reveal an underlying pattern of bilateral behavior between forebrain and cardiovascular system uncovering new aspects of neuroendocrine asymmetries whose functional meaning remains to be elucidated.

The FC is involved in the control of cognitive and cardiovascular functions [73] this connection being lateralized [75]. The brain RAS is involved in the regulation of behavior [20]. Captopril modifies the bilateral distribution of AG in FC [70] and improves cognition and depressed mood [76, 77]. In a model of myocardial infarction in rats, captopril therapy appeared to decrease anxiety in the infracted group but increase anxiety in the sham-operated rats. This response, however, differed depending on the test used to measure anxiety [77]. It has also been reported that the ACEI captopril and enalapril improve cognition and depressed mood in hypertensive patients [76]. Modifications in the basal FC asymmetries have been associated with changes in behavior, including emotional state [78]. Therefore, the bilateral changes observed in the correlations of AG between the cardiovascular system and FC after captopril treatment, presumably modulated by the autonomic nervous system [70, 74], might also be involved in the changes of the emotional state.

Treatment with ARB also modifies the RAS cascade at several levels suggesting that the effects of these compounds may also be the consequence of such changes in RAS. For example, it has been reported that Ang 1-7 contributes to the cardiovascular effects of ARB in both animals and humans [37]. The ARB telmisartan induced an increase in the myocardial levels of ACE_2 and Mas-receptor in animals with experimental autoimmune myocarditis suggesting that telmisartan protect against heart failure in rats, at least in part by modulation of ACE_2/ANG1-7/Mas receptor axis [79]. Agata *et al.* [80] suggested that olmesartan, another ARB, in addition to block the AT_1 receptor may inhibit ACE and protect from cardiovascular remodeling through increased local production of NO and Ang 1-7 in the myocardium following increased expression of ACE_2. Moreover, it was suggested that the beneficial effects of the ARB telmisartan and losartan in adriamycin-induced heart failure in rats may be partly due to the increase in plasma levels of Ang 1-7 and to the decrease in myocardial AT_1 expression. However, in these experiments, no modifications in the expression of both Mas receptor and AT_2 were observed in the myocardium [81]. On the other hand, the metabolism of Ang III to Ang IV by AP M and of Ang I to Ang 2-10 by AspAP was analyzed in the renal cortex and medulla of normotensive (sham-operated) and hypertensive Goldblatt two-kidney one clip (G2K1C) rats, treated or not with the ARB valsartan [44]. The results demonstrated a highly significant increase of AP M only in the cortex of the non-ischemic kidney of G2K1C rats treated with vehicle and valsartan, compared with the kidney of normal rats or with the clipped kidney of G2K1C rats treated or not with valsartan.

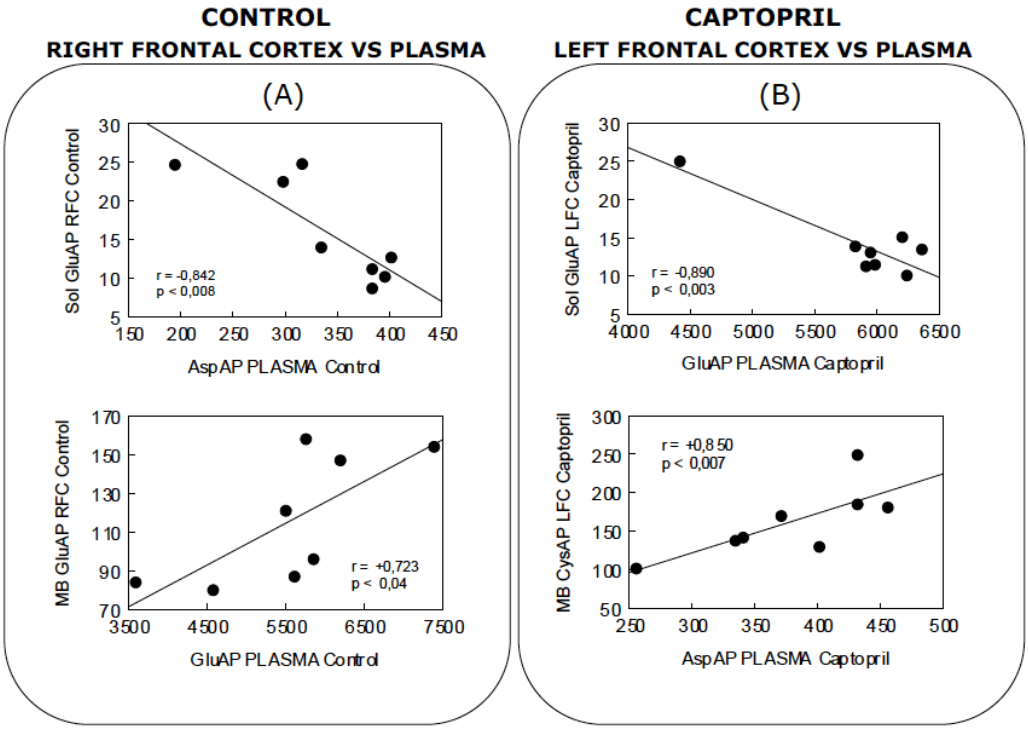

Fig. (3). Correlations between the right (**A**) or left (**B**) frontal cortex soluble (Sol) or membrane-bound (MB) aminopeptidase activities versus plasma aminopeptidase activities in the control (**A**) and captopril-treated animals (**B**). Pearson's correlation coefficients (r) and p-values are indicated and specify the significance of the differences between these correlations [70] (see text for abbreviations).

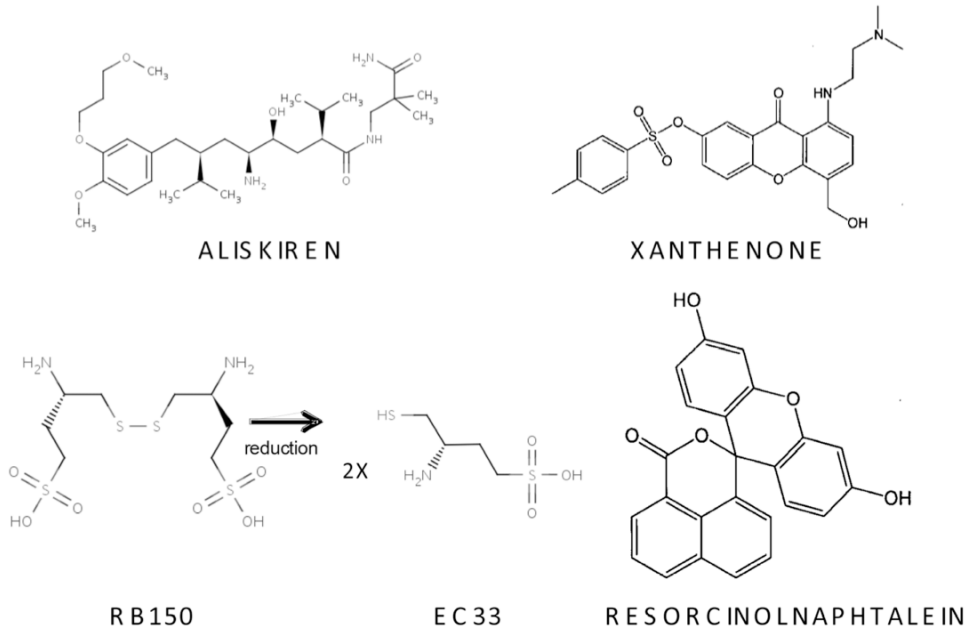

Fig. (4). Chemical structures of some recently developed compounds influencing on angiotensinases. Aliskiren inhibits renin [62]. RB150 is a prodrug of EC33, an inhibitor of AP A [13]. Xantenone and Resorcinolnaphtalein have been developed as activators of ACE_2 [42].

This suggested an increased formation of Ang IV in the non-clipped kidney of G2K1C rats. It was demonstrated that the administration of Ang IV through the renal artery produced a dose-related increase in cortical blood flow without modification of systemic BP, this effect being dependent of the NO release from endothelial cells [30]. In addition, in G2K1C rats, NO may play an important role in systemic and non-clipped kidney hemodynamics [82], which might be due to the presumably increased formation of Ang IV, caused by the increase of AP M in the non-ischemic (non-clipped) kidney [44]. In addition, in these experiments, valsartan reduced AP M and AspAP activities in the renal cortex of normoten-

Table 1. Summary of Characteristics and Properties of Some Angiotensinases

AG	E.C. Number	Catalyzed Reaction in the RAS	Chemical Action on the Enzyme	Clinical Application
Renin	3.4.23.15	AOGEN to Ang I	Inhibition	Reduction of BP blocking the RAS cascade [62]
ACE	3.4.15.1	Ang I to Ang II	Inhibition	Reduction of BP and cardioprotective [16]. Attenuation of end-organ damage [17-19]. Improvement of insulin sensitivity and glucose homeostasis [66]. Improvement of cognition and depressed mood [76, 77]
ACE_2	3.4.17.23	Ang I to Ang 1-9 Ang II to Ang 1-7	Activation	Reduction of BP, improves cardiac function and reverses myocardial fibrosis [39, 42]. Gastroprotective [40]. Protective in respiratory distress syndrome [41].
GluAP (AP A)	3.4.11.7	Ang II to Ang III	Inhibition	Reduction of BP by brain inhibition [13]
AspAP	3.4.11.21	Ang I to Ang 2-10	Activation*	Protective vascular actions in kidney and mesenteric beds [44, 45]. Counterbalance Ang II and Ang III in brain [46, 64]. Improve glucose tolerance and insulin sensitivity [47]

*Still under research.

sive and in the clipped kidney of hypertensive rats. The reduced metabolism of Ang III may prolong its half-life in valsartan-treated animals. Therefore, in spite of the well-known contribution of Ang II in the pathogenesis of G2K1C hypertension, the importance of other Ang peptides, such as Ang III, Ang IV or Ang 2-10, should also be considered.

Other antihypertensive drugs such as the peripheral sympathetic beta-blockers may also influence the RAS at several levels. For example, the treatment of cirrhotic patients with propranolol resulted in a significant reduction of plasma renin activity, Ang I, Ang II and Ang 1-7 in both the portal vein and the peripheral circulation [83]. In order to analyze whether Ang 1-7 is a protective peptide against renal injury, Igase *et al.* [84] treated SHR with the AT_1 antagonist olmesartan or with the beta blocker atenolol. After treatment, plasma Ang 1-7 was significantly higher in animals treated with olmesartan than atenolol or vehicle and the peptide correlated negatively with the glomerulosclerosis index suggesting that Ang 1-7 may play a role in preventing hypertension-induced renal injury. Finally, preliminary results suggested that after propranolol treatment of SHR there was a significant increase in the formation of Ang III and Ang 2-10 in plasma, indicating that these peptides may also influence the beneficial effects of the beta-blockers [85].

CONCLUDING REMARKS

Traditionally, Ang II is considered the main peptide of the RAS, involved in the control of BP and hydroelectrolytic balance. However, it has become obvious that it is involved in a number of other processes, including brain functions and that other peptides of the system, such as Ang III, Ang IV, Ang 1-9, Ang 1-7, or Ang 2-10, seem also to play an important role in the pathophysiological functions of the RAS. The role of some angiotensinases is summarized in (Table **1**). The use of the classical antihypertensive drugs, currently available, such as ACEI, ARB or beta-blockers, may influence the RAS at several levels of the cascade, promoting or inhibiting the formation of specific Ang peptides other than Ang II. Currently, there are increasing evidences that the beneficial effects of these drugs may be not only the consequence of their blockade of Ang II formation/binding or of their inhibition of sympathetic actions, but also the result of the increased formation of other Ang peptides that may counteract the detrimental actions of Ang II. However, such modification in the RAS may have also other unexpected consequences, for instance changes in brain bilateral functions, which could not necessarily be beneficial.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

Supported by Ministry of Science and Innovation through project no.: SAF-2008 04685 C02.

ABBREVIATIONS

ACE = Angiotensin-Converting Enzyme

ACEI = Angiotensin-Converting Enzyme Inhibitors

ADH = Vasopressin

AG = Angiotensinases

A-LAP = Adipocyte-Derived Leucine Aminopeptidase

Ang = Angiotensin

AOGEN = Angiotensinogen

AP A = Aminopeptidase A

AP B = Arginyl-Aminopeptidase

AP M = Alanyl-Aminopeptidase

AP N	=	Alanyl-Aminopeptidase
ARB	=	Angiotensin Receptor Blockers
AspAP	=	Aspartyl-Aminopeptidase
BP	=	Blood Pressure
Carb-P	=	Carboxipeptidase P
G2K1C	=	Goldblatt Two-Kidney One Clip
GluAP	=	Glutamyl-Aminopeptidase
IRAP	=	Insulin-Regulated Aminopeptidase
NEP	=	Neutral-Endopeptidase
NO	=	Nitric Oxide
PRR	=	Prorenin
PRR-R	=	Prorenin Receptor
RAS	=	Renin-Angiotensin System
SHR	=	Spontaneously Hypertensive Rats

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Received: ????? **Revised: ?????** **Accepted: ?????**