Antihypertensive effects of angiotensin-(1-7)

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Abstract

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Accumulating evidence suggests that angiotensin-(1-7) (Ang-(1-7)) is an important component of the renin-angiotensin system and that the actions of the peptide may either contribute to or oppose those of Ang II. Ang-(1-7) can be converted directly from Ang I bypassing prerequisite formation of Ang II. Formation of Ang-(1-7) is under the control of at least three endopeptidases depending on the tissue compartment and include neprilysin, thimet oligopeptidase and prolyl oligopeptidase. Both neprilysin and thimet oligopeptidase are also involved in the metabolism of bradykinin and the atrial natriuretic peptide. Moreover, recent studies suggest that in addition to Ang I and bradykinin, Ang-(1-7) is an endogenous substrate for angiotensin converting enzyme. These enzymatic pathways may contribute to a complex relationship between the hypertensive actions of Ang II and various vasodepressor peptides from either the renin-angiotensin system or other peptide systems. Ang-(1-7) is devoid of the vasoconstrictor, central pressor, or thirst-stimulating actions associated with Ang II. In fact, new findings reveal depressor, vasodilator, and antihypertensive actions that may be more apparent in hypertensive animals or humans. Thus, Ang-(1-7) may oppose the actions of Ang II directly or as a result of increasing prostaglandins or nitric oxide. In this review, we examine the mechanisms by which Ang-(1-7) may contribute to cardiovascular regulation.

Introduction

It is well recognized that the renin-angiotensin system plays an important role in the overall control of water and salt homeostasis. The generation of angiotensin II (Ang II) from angiotensin I (Ang I) has long been considered the final product of this system. Ang II induces a variety of actions in the vasculature, brain, pituitary, adrenal and kidney to augment blood pressure. These actions include vasoconstriction, cellular hypertrophy, salt and water retention and stimulation of drinking. Indeed, the inhibition of the renin angiotensin system has been shown to be a powerful strategy to lower blood pressure in the clinical setting.

Recent studies have revived the possibility that angiotensin peptides other than Ang II may either contribute to or actually oppose the cardiovascular actions of Ang II, endowing this hormonal system with a greater flex-

ibility than originally imagined. The characterization of angiotensin-(1-7) (Ang-(1-7)) as the first amino terminal angiotensin peptide product possessing biological actions provided a foundation for the pursuit of a new concept regarding the regulation of cardiovascular function by the renin-angiotensin system. The accumulating evidence suggests that Ang-(1-7) may serve to counterbalance the actions of Ang II (1). This review updates the progress that has been made in the development of this concept by examining the diverse actions of Ang-(1-7) and the receptors that mediate these actions.

Pathways of angiotensin-(1-7) formation and degradation

Illustrated in Figure 1 are the major bioactive components of the renin-angiotensin system. As shown, the enzymatic cascade diverges with the processing of Ang I to either Ang II via converting enzyme (ACE) or to Ang-(1-7). Ang II is then N-terminally metabolized to the smaller bioactive fragments Ang-(2-8) and Ang-(3-8) by peptidyl (AP) or dipeptidyl (DAP) aminopeptidases.

Conversely, Ang I can be directly converted to Ang-(1-7) bypassing formation of Ang II (2). This pathway is potentially under the control of three endopeptidases which form Ang-(1-7) including neprilysin (NEP), thimet oligopeptidase (TO) and prolyl oligopeptidase (PO) (2), as well as ACE. Although the generation of Ang-(1-7) from Ang II has not been fully investigated, PO and prolyl carboxypeptidase (PCP) both cleave the Pro^7-Phe^8 bond of Ang II.

The biosynthetic pathways for the formation of Ang-(1-7) from Ang I are well understood; however, the route for the degradation of the peptide has not been elucidated. Several mechanisms have been postulated for the removal of peptides from the circulation including receptor-mediated processes (3,4) and enzymatic metabolism. We and others have recently shown that Ang-(1-7) is hydrolyzed to Ang-(1-5) by ACE *in vitro* (5,6). Initial evidence that Ang-(1-7) was a substrate for ACE arose from a comparison of the potencies of various angiotensin and bradykinin peptides to inhibit ACE activity. Ang-(1-7) was a more potent competitor of ACE than bradykinin, Ang I or substance P

Figure 1 - Pathways for the generation and metabolism of angiotensin peptides. Angiotensin I (Ang I) is processed to biologically active products through distinct enzymatic pathways. Ang II is formed by the hydrolysis of the Phe^8-His^9 bond of Ang I by angiotensin converting enzyme (ACE). Ang II is further processed by aminopeptidases (AP) or dipeptidyl aminopeptidases (DAP) to yield the active metabolites Ang-(2-8) and Ang-(3-8). Ang-(1-7) is formed by the hydrolysis at Pro^7-Phe^8 of Ang I by several endopeptidases including neprilysin (NEP), thimet oligopeptidase (TO) and prolyl oligopeptidase (PO) and from Ang II by PO and prolyl carboxypeptidase (PCP). Ang-(1-7) is hydrolyzed at the Ile^5-His^6 bond by ACE to yield Ang-(1-5); Ang II is cleaved at Tyr^4-Ile^5 by NEP to yield Ang-(1-4).
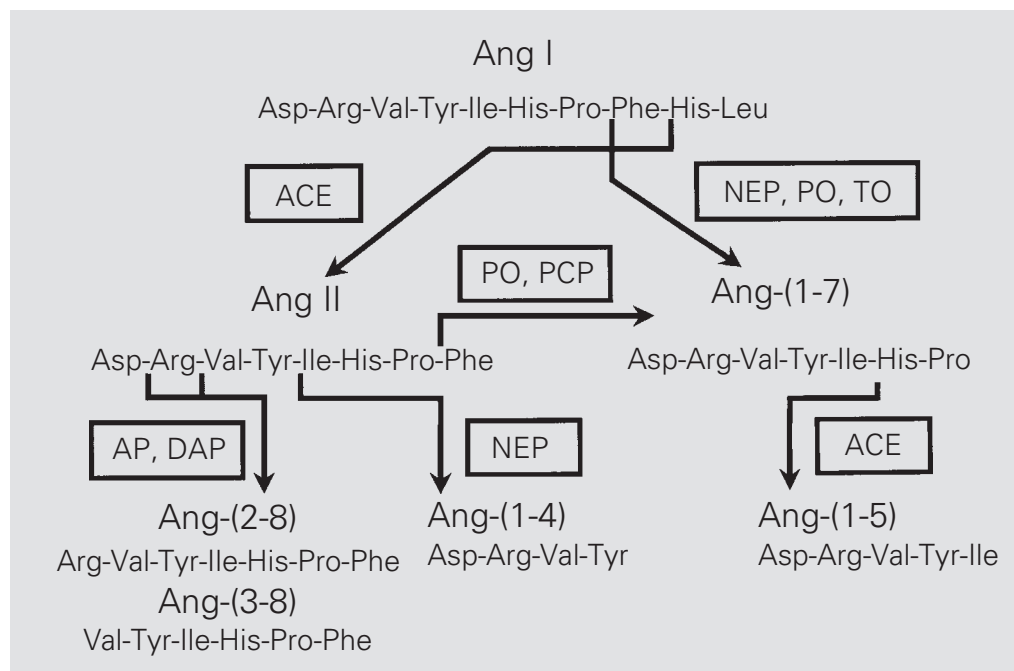
(Figure 2, top panel) (5,7). Interestingly, the Ang-(1-7) antagonist D-[Ala⁷]-Ang-(1-7) in which the carboxyl-terminal or C proline is substituted with D-alanine did not compete for activity (Figure 2) nor was the peptide hydrolyzed by ACE (Chappell MC, unpublished results). The complete inhibition with di-nitrofluorobenzene (DNFB, Figure 2, middle panel) and the greater potency of lisinopril over captopril (2.1 nM *vs* 27 nM, respectively; Figure 2, bottom panel) suggest that the C domain of ACE is primarily involved in the hydrolysis of Ang-(1-7) (8,9). However, Deddish et al. (6) have recently reported that Ang-(1-7) is a selective substrate for the amino or N domain of human ACE and may inhibit the C domain. Although both canine and human ACE exhibited similar kinetic values for Ang-(1-7), there may exist species differences concerning which domain participates in the hydrolysis of the peptide. The favorable kinetic constants (Km = 0.8 μM, kcat = 1.8/s for canine ACE) suggest an *in vivo* role for ACE in the regulation of Ang-(1-7) and recent studies demonstrate that lisinopril augments the half-life of infused Ang-(1-7) by 4- to 5-fold (10). Thus, the marked increase in circulating levels of Ang-(1-7) following chronic ACE inhibition reflects both increased synthesis (due to higher Ang I levels) and decreased metabolism.

Physiological actions of angiotensin-(1-7)

There is substantial evidence available now to demonstrate that the fragments formed from Ang I and Ang II metabolism are biologically active. Of the biologically active fragments studied to-date, the physiological actions of Ang-(1-7) have been most widely investigated. This peptide has been shown to be present in the plasma and a variety of tissues in both humans and rats. Ang-(1-7) elicits physiological effects that are similar or opposite to that of Ang II. In the brain,

Ang-(1-7) stimulates release of vasopressin (11,12) and facilitates baroreflexes (13-15). However, unlike Ang II, the peptide does not stimulate dipsogenesis (16) or elicit potent vasoconstrictor actions. At the cellular level, Ang-(1-7) stimulates release of PGE_2 and PGI_2 (17-21), potentiates the hypotensive effects of bradykinin (7,22-25), and stimulates the release of nitric oxide (7,24,26).
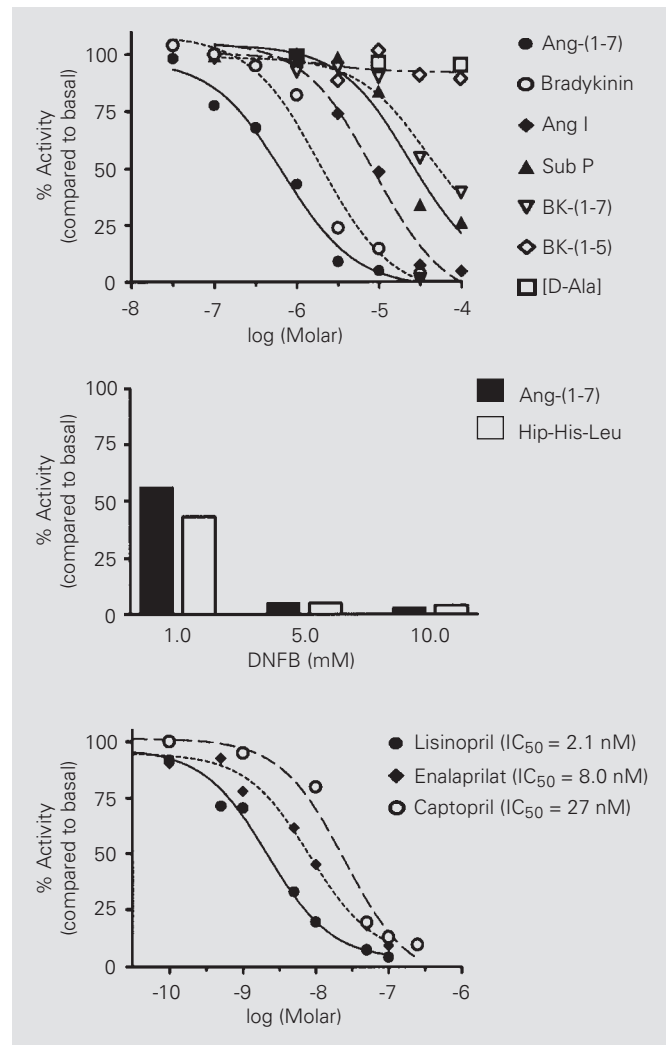

Figure 2 - *Top panel*, Comparison of the potencies of angiotensin and bradykinin peptides to compete for ACE activity determined with the synthetic substrate Hip-His-Leu as described (5). Sub P, Substance P; Ang, angiotensin; BK, bradykinin; [D-Ala], D-[Ala⁷]-Ang-(1-7). *Middle panel*, Effects of di-nitrofluorobenzene (DNFB) on the ACE-dependent hydrolysis of Ang-(1-7) and Hip-His-Leu. *Lower panel*, Potencies of the inhibitors lisinopril, enalaprilat and captopril to inhibit the hydrolysis of Ang-(1-7) by ACE.

Figure 3 - Strategy for the blockade of peripheral Ang-(1-7). Circulating angiotensin I (Ang I) is hydrolyzed to Ang-(1-7) by neprilysin (NEP) situated on the vascular surface. Attenuation of the vasodilator actions of Ang-(1-7) is achieved by either inhibiting the synthesis of the peptide with a neprilysin inhibitor (NEPI), neutralization of the circulating peptide with monoclonal antibody (mAb-Ang-(1-7)) or blockade of the putative receptor site with the non-selective antagonist [Sar[1],Thr[8]]-Ang II (Sarthran).
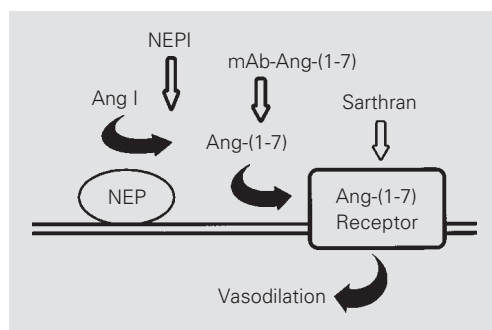
Finally Ang-(1-7) exerts a vasodilatory effect which may account for its antihypertensive effects that are manifested *in vivo* (19,27,28). The fact that the physiological effects of Ang-(1-7) are either identical or opposite to those of Ang II indicates that this is a pleiotropic fragment. Collectively, the various physiological effects of Ang-(1-7) mentioned above would favor a blood pressure lowering effect under conditions of high Ang II activity.

The biological function of the enzymes forming Ang-(1-7) reinforces the idea that this peptide is a component of a vasodepressor system regulating blood pressure. The two enzymes, neprilysin and thimet oligopeptidase that have been shown to form Ang-(1-7) from Ang I (2,29) also cleave bradykinin and the atrial natriuretic peptide to smaller fragments (30). These observations suggest that the various angiotensin products and other vasodepressor peptides are intertwined through these enzymatic pathways, and this is a concept of importance. Physiologists have always considered that Ang II could indirectly lead to activation of vasodepressor systems. However, the fact that Ang-(1-7) may exhibit antihypertensive actions and arises from Ang I points to its existence within the renin angiotensin system itself for mitigation of the actions of Ang II. Thus, the role of the smaller fragments of the renin angiotensin system in physiology and pathology should not be examined independently from Ang II.

The relationship between the status of the renin angiotensin system and the antihy-

pertensive response to ACE inhibitors or Ang II antagonists is not a simple one. Often, these agents show good antihypertensive activity in the presence of normal or even suppressed renin activity (31,32). The argument has been posed that the chronic antihypertensive action of ACE inhibitors may be mediated by accumulation of tissue bradykinin (33). However, Cachofeiro et al. (34) reported that, in contrast to acute lisinopril treatment, the kinin B2 antagonist HOE 140 did not reverse the antihypertensive effects of chronic lisinopril treatment. We and others have reported similar observations in spontaneously hypertensive rats (SHR) treated with lisinopril/losartan and in renal hypertensive rats with ramipril treatment (35,36).

In this regard, we began a series of studies to determine whether Ang-(1-7) contributes to the antihypertensive effects of a combined lisinopril/losartan regimen in SHR. As shown in Figure 3, three strategies were employed to attenuate the potential actions of Ang-(1-7): 1) inhibition of Ang-(1-7) formation with a neprilysin inhibitor; 2) neutralization of the peptide by infusion of a selective monoclonal antibody (mAb-Ang-(1-7)), and 3) blockade of the receptor with the non-selective antagonist [Sar[1],Thr[8]]-Ang II (Sarthran). The combined treatment with an ACE inhibitor and AT_1 antagonist should favor the conversion of Ang I to Ang-(1-7) by neprilysin (37) and block actions at the AT_1 receptor. Systemic administration of an Ang-(1-7) monoclonal antibody at increasing concentrations partially reversed the antihypertensive response in SHR chronically treated with lisinopril/losartan (Figure 4, top panel) (38). Acute inhibition of the endogenous synthesis of Ang-(1-7) by two different neprilysin inhibitors (SCH 39370 and CGS 24592) resulted in a similar reversal of the antihypertensive effect produced by the lisinopril/losartan in SHR (Figure 4, lower panel) (36). The increase in blood pressure with the neprilysin inhibitor CGS 24592 was

associated with a 60% fall in the plasma concentrations of Ang-(1-7) (36). Administration of Sarthran also induced a pressor response in the SHR that was not attenuated by prior blockade with the AT_2 antagonist PD 123319 and indicates that the vasodepressor actions of Ang-(1-7) are mediated via a non-AT_1/AT_2 receptor. In addition, prior treatment with Sarthran prevented any further increase in blood pressure with the CGS compound (Figure 4). These data suggest that the effects of ACE inhibitors may be partially mediated by Ang-(1-7) and add a new and important dimension to the understanding of the physiology of the renin angiotensin system.

Receptors mediating the actions of ang-(1-7)

Accumulating evidence suggests that the effects of Ang-(1-7) are mediated by a unique angiotensin receptor (1,38). The stimulation of prostaglandin E_2 and I_2 synthesis, and nitric oxide release by Ang-(1-7) occur via activation of a receptor subtype distinct from AT_1 and AT_2 but recognized by the competitive non-selective Ang II antagonist Sarthran (1). Similarly, the *in vivo* vasodepressor effects of Ang-(1-7) have been shown to be mediated, in part, by non-AT_1/AT_2 receptor subtypes that are sensitive to Sarthran (38). In addition, a high affinity binding site has been described in bovine endothelial cells in culture (39) and canine coronary artery endothelium by *in vitro* autoradiography (1). Thus, the majority of the data available suggest that Ang-(1-7) may act at a novel non-AT_1/AT_2 receptor, the signal transduction pathway for which still remains to be elucidated. However, it should be noted that, under certain conditions, the effects of Ang-(1-7) may be blocked by losartan or to a variable extent by AT_2 receptor antagonists (17,18,40), suggesting a heterogeneity of Ang-(1-7) receptors sensitive to either AT_1 or AT_2 antagonists. This may be particularly
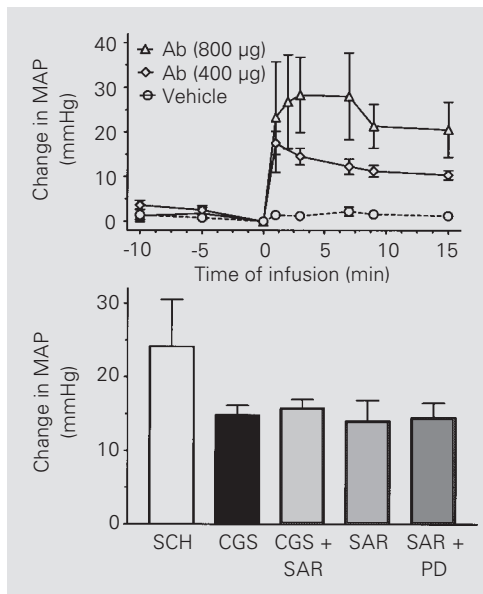

Figure 4 - *Top panel*, Peripheral administration of an Ang-(1-7) monoclonal antibody (Ab) increases mean arterial blood pressure (MAP) in chronically treated losartan/lisinopril spontaneously hypertensive rats (SHR) as described (38). Vehicle administration (Vehicle; normal mouse IgG, 400 µg/min) did not affect MAP. *Bottom panel*, Effect of neprilysin (NEP) inhibitors and angiotensin receptor antagonists on MAP from the losartan/lisinopril-treated SHR (36). Animals were acutely treated with the NEP inhibitors SCH 39370 (SCH), CGS 24592 (CGS) or the antagonists [Sar[1],Thr[8]]-Ang II (SAR) or PD 123319 (PD).

evident regarding the actions of Ang-(1-7) in the kidney (see below for further discussion of sites within the kidney).

Renal actions of angiotensin-(1-7)

Renal infusion of Ang-(1-7) produced marked diuresis and natriuresis in the isolated (41) and intact kidney of Sprague Dawley (SD) and Wistar rats, respectively (42,43). In contrast to the potent renal actions of Ang II, Ang-(1-7) lacked any effect on renal blood flow and tended to increase the glomerular filtration rate (41,43). The diuretic actions of Ang-(1-7) in the isolated kidney were attenuated by the cyclooxygenase inhibitor indomethacin (44). In cultured renal tubular epithelial cells from rabbit, Ang-(1-7) inhibited transcellular sodium flux (45). Interestingly, the inhibition of sodium transport with Ang I was markedly potentiated by the ACE inhibitor captopril. The brush border of proximal tubules contains high concentrations of neprilysin, an endopeptidase which cleaves Ang I directly to Ang-(1-7) (46). Ang-(1-7) also inhibited transport-dependent oxygen consumption, a marker for Na-K-ATPase activity in isolated convoluted proximal tubules (43). This po-

tent inhibition by Ang-(1-7) was partially blocked by an AT_1 antagonist and completely attenuated by Sarthran while the AT_2 antagonist PD 123319 had no effect. However, Ang-(1-7) exhibited biphasic effects on water and bicarbonate transport in perfused straight proximal tubules (47). A low concentration (1 pM) of Ang-(1-7) stimulated water transport, while 10 nM inhibited fluid absorption most probably by altering the Na^+/H^+ exchanger. The biphasic actions of Ang-(1-7) were completely blocked by losartan and the AT_2 antagonist had no effect (47).

The effect of various angiotensin antagonists and the tubular actions of Ang-(1-7) suggest that this peptide may distinguish multiple AT_1 receptor sites in the kidney. Santos and colleagues also report that Ang-(1-7) may interact with a novel AT_1 or losartan-sensitive site in the kidney (12). Ang-(1-7) promotes an anti-diuretic action in water-loaded Wistar rats with a tendency for increased plasma vasopressin (12,48). Both the AT_1 agent losartan and the Ang-(1-7) antagonist D-[Ala[7]]-Ang-(1-7) attenuated the anti-diuretic effects of Ang-(1-7) (49). The D-[Ala[7]] antagonist has also been reported to block the inhibition of water transport by Ang-(1-7) in a collecting duct preparation (12). The D-[Ala[7]]-Ang-(1-7) compound does not inhibit Ang II binding at typical AT_1 or AT_2 sites in the adrenal or attenuate the vasoconstrictor effects of Ang II (50).

The intriguing actions of Ang-(1-7) to inhibit diuresis are completely opposed to the effects observed in the perfused kidney and deserve further comment. One obvious difference in the studies with intact animals was that the experiments were performed after water loading. In human patients, differential effects were observed with AT_1 treatment following an acute water load (51). Importantly, these data emphasize that the overall state of sodium and water balance, as well as the overall activity of the renin-angiotensin system may greatly influence the effects of Ang-(1-7) in the kidney. Perhaps of equal importance, the dose of the peptide, the route of administration and the site of the nephron exposed to Ang-(1-7) may also influence the actions of the peptide.

Conclusions

The angiotensin fragments of the renin angiotensin system cascade have been shown to possess biological activity although their role in the maintenance of the physiological process is still not clear. Of all these metabolites, Ang-(1-7) may be the most pleiotropic as it exerts effects that either oppose those of Ang II or comprise a subset of Ang II actions. The studies reported above provide a new understanding to the contribution of the renin angiotensin system in physiology and pathology.

Acknowledgments

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