Novel Angiotensin Peptides Regulate Blood Pressure, Endothelial Function, and Natriuresis

CARLOS M. FERRARIO, MARK C. CHAPPELL, RICHARD H DEAN, and SHRIDHAR N. IYER

The Hypertension and Vascular Disease Center, Wake Forest University School of Medicine, Winston-Salem, North Carolina.

Abstract. Accumulating evidence suggests that angiotensin-(1-7) is an important component of the renin-angiotensin system, having actions that are either identical to or opposite that of angiotensin II. Angiotensin I can be directly converted to angiotensin-(1-7), bypassing formation of angiotensin II. This pathway is under the control of three enzymes: neutral endopeptidases 24.11 (neprilysin) and 24.15 and prolyl-endopeptidase 24.26. Two of the three angiotensin-forming enzymes (neprilysin and endopeptidase 24.15) also contribute to the breakdown of bradykinin and the atrial natriuretic peptide. Furthermore, angiotensin-(1-7) is a major substrate for angiotensin-converting enzyme. These observations suggest that the process of biotransformation between the various Ang peptides of the renin-angiotensin system and other vasodepressor peptides are intertwined through this enzymatic pathway. Substantial evidence suggests that angiotensin-(1-7) stimulates the synthesis and release of vasodilator prostaglandins, and nitric oxide, while also augmenting the metabolic actions of bradykinin. In addition, angiotensin-(1-7) alters tubular sodium and bicarbonate reabsorption, decreases Na+-K+-ATPase activity, induces diuresis, and exerts a vasodilator effect. These physiologic effects of angiotensin-(1-7) favor a blood pressure-lowering effect. The majority of the data currently available suggest that angiotensin-(1-7) mediates its effects through a novel non-AT_{1}/AT_{2} receptor subtype.

Evidence suggests that peptidergic hormones may be cleaved into alternate smaller fragments, which remain biologically active by interacting with native or congener receptor subtypes. This process of biotransformation, well known to neuroendocrinologists (1), has only recently attracted the attention of those who labor to unveil the function of hormones regulating blood pressure. The renin-angiotensin system (RAS) is clearly an example of a peptidergic hormone system in which precursor peptides are transformed into active products through a stepwise enzymatic processing assumed to sequentially convert angiotensinogen into the active product angiotensin II (AngII). As recently reviewed by Ardaillou (2), this assumption needs to be reconsidered. At least three other angiotensin peptides have been shown to possess biological activity. Their contribution to the long-term regulation of homeostasis and cardiovascular pathology has not been fully explored, primarily because of the ubiquitous actions of AngII. Angiotensin III [Ang-(2-8)] was recognized early to be a potent aldosterone secretagogue, whereas angiotensin IV [Ang-(3-8)] and angiotensin-(1-7) [Ang-(1-7)] have a more recent history. Altogether, the demonstration that AngI and AngII are processed into other active fragments suggests that the biochemical cascade by which the RAS exerts its biological actions is more complex than originally envisioned (3). This review focuses on the physiologic actions of Ang-(1-7) and receptors mediating these effects. In addition, the renal effects and pathways mediating the formation and degradation of Ang-(1-7) are discussed. These new studies suggest that Ang-(1-7) may contribute to the regulation of blood pressure by counterbalancing the effects of AngII, a finding that imbues the RAS with the intrinsic capacity to inhibit the pressor and trophic actions of the native hormone.

Production of Angiotensin-(1-7)

Ang-(1-7) differs from AngII by the absence of an amino acid at the carboxy terminus due to the removal of the peptide bond between the seventh (proline) and eighth (phenylalanine) residue. Earlier studies of the structure-activity relationship of AngII suggested that the presence of phenylalanine of the molecule was critical for biological activity (4). Because Ang-(1-7) is neither a vasoconstrictor nor an aldosterone secretagogue (5), the heptapeptide joined the family of AngII fragments considered to represent inactive products of the metabolic degradation of the native hormone.

Less than a decade ago, Ang-(1-7) was first observed to be an active fragment of the angiotensin system (6) and to be cleaved from either AngI or AngII by specific tissue endopeptidases. Figure 1 illustrates schematically a revised pathway for the biochemical processing of AngI into the major bioactive components of the RAS. AngI, formed from angiotensinogen
Angiotensin I (AngI) is processed to biologically active products through distinct enzymatic pathways. AngII is formed by the hydrolysis of the Phe⁵-His⁹ bond of AngI by angiotensin-converting enzyme (ACE). AngII 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 AngI by endopeptidases (ENDOPEP) neprilysin (NEP) and prolyl endopeptidase (PE). AngII may be converted to Ang-(1-7) by carboxypeptidases (CARBOXYPEP) PE and prolyl carboxypeptidase (PCP). Ang-(1-7) is hydrolyzed at Ile⁵-His⁶ by ACE to yield Ang-(1-5); AngII is cleaved at Tyr⁴-Ile⁵ by NEP to yield Ang-(1-4).

Although the biosynthetic pathway for the formation of Ang-(1-7) is now well understood, the enzymes accounting for the inactivation of Ang-(1-7) were assumed to belong to the family of tissue aminopeptidases (11). Recent studies suggest that ACE hydrolyzes Ang-(1-7) into the pentapeptide angiotensin-(1-5) (12), a process that may account for the remarkably short half-life of Ang-(1-7) (<10 seconds) in the bloodstream (13). The role of ACE in determining both the disposition and activity of Ang-(1-7) may be more important than originally considered; Deddish *et al.* (14,15) observed that the heptapeptide acts as an endogenous inhibitor of the carboxyl (C) domain of somatic ACE.

**Actions of Angiotensin-(1-7)**

Ang-(1-7) may counterbalance the effects of AngII in some tissues, while it may mimic the actions of AngII in others. In the brain, Ang-(1-7), similar to AngII, stimulates the release of vasopressin (14-17). However, Ang-(1-7) does not stimulate thirst (18) or inhibit the baroreceptor reflex (19-21). Within the kidney, Ang-(1-7) alters tubular sodium and bicarbonate reabsorption (22-24), decreases Na⁺-K⁺-ATPase activity (23), and induces diuresis and natriuresis (23,25-27). At the cellular level, Ang-(1-7) stimulates release of prostaglandins E₂ and I₂ (28-32), augments the vasodilator effects of bradykinin (12,33-36), and stimulates the release of nitric oxide. Finally, on the vasculature, Ang-(1-7) exerts a vasodilator effect, which may account for its antihypertensive effects (37-39). The fact that Ang-(1-7) exerts physiologic effects that are either identical to or opposite that of AngII makes it a pleiotropic fragment. Collectively, the various physiologic actions of Ang-(1-7) may account for its antihypertensive effects (37-39). 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 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. Two of the three Ang-(1-7)-forming enzymes, neprilysin (NEP 24.11) and endopeptidase 24.15, also cleave bradykinin and the atrial natriuretic peptide to smaller fragments (40). These observations suggest a functional intricacy among enzymes that may influence the fate of potent vasodilator peptides. This is an important concept. Abundant literature suggests that the pressor actions of AngII can be restricted by the peptide effect on stimulation of vasodilator prostaglandins (41,42), nitric oxide (43,44), and the actions of AngII at the AT₂ receptor subtype...
Although these interactions are important, our studies suggest the additional existence within the RAS of a mechanism for limiting the pressor actions of AngII through the counterbalancing actions of Ang-(1-7) (3). In this context, the physiologic and pathologic roles of Ang-(1-7) cannot be examined independently from those of AngII.

The relationship between the status of the RAS and the antihypertensive response to ACE inhibitors or AngII blockers is not a simple one. Often, these agents show good antihypertensive activity in the presence of normal or even suppressed renin activity (47,48). The argument has been posed that the long-term antihypertensive action of ACE inhibitors may be mediated by accumulation of tissue bradykinin (49,50). However, Cachofeiro et al. (51) reported that long treatment with the specific B2 receptor bradykinin antagonist HOE 140 did not reverse the antihypertensive effects of ACE. Similar observations were reported by Bao et al. (52) and in our recent studies (53). Thus, other mechanisms may be involved in mediating the antihypertensive effects of ACE inhibitors. An important finding from both clinical (54) and experimental studies (55,56) is that chronic inhibition of ACE is associated with significant elevation in plasma Ang-(1-7). The antihypertensive effects of a combined lisinopril and bosartan treatment were reversed partially using an Ang-(1-7) monoclonal antibody (57) or the administration of a specific inhibitor of neprilysin (53). These data suggest that the effects of ACE inhibitors may be partially mediated by Ang-(1-7), adding a new and important dimension to the understanding of the physiology of the RAS.

**Receptors Mediating the Actions of Angiotensin-(1-7)**

New studies suggest that Ang-(1-7) mediates its effects through a unique Ang receptor (3,57). Stimulation of prostaglandin E2 and I3 synthesis, and nitric oxide release by Ang-(1-7) occur via activation of a receptor subtype distinct from AT1 and AT2 but recognized by the competitive nonselective AngII antagonist [Sar'-Thr8]-AngII (3). Similarly in vivo, the vasodepressor effects of Ang-(1-7) are mediated via non-AT1/AT2 receptor subtypes that are sensitive to [Sar'-Thr8]-AngII (57). However, the effects of Ang-(1-7) may be blocked by high concentrations of losartan or to a variable extent by AT2 receptor antagonists under certain conditions. Since the haptedopeptide does not mimic the effects of AngII at these sites, the studies suggest a heterogeneous population of Ang-(1-7) receptors (28,29,57). A high affinity-binding site has been described in bovine endothelial cells in culture (58) and canine coronary artery endothelium by in vitro autoradiography (3). Thus, the majority of the data available currently suggest that Ang-(1-7) may act at a novel non-AT1/AT2 receptor subtype, the signal transduction pathway for which still remains to be elucidated.

**Antihypertensive Actions of Angiotensin-(1-7)**

Ang-(1-7) occurs in the plasma and tissues of several species, including humans (3). Chronic treatment with ACE inhibitors elevates plasma concentrations of Ang-(1-7) in humans (60) and rats (54), whereas urinary excretion rates of Ang-(1-7) are low in untreated essential hypertensive subjects (61) and in the plasma of spontaneously hypertensive rats (SHR) (54). Even in [mRen-2]27 transgenic hypertensive rats (Tg+) there is a reversal of the plasma and brain tissue ratios of Ang-(1-7)/AngII (62).

When administered intravenously, Ang-(1-7) elicits a biphasic response consisting of an initial and rapid pressor response mediated via the AT1 receptors, followed by a prolonged depressor response mediated via a non-AT1/AT2 receptor (39). The depressor component of Ang-(1-7) was abolished by pretreatment with [Sar'-Thr8]-AngII, a nonselective Ang antagonist, and not by losartan, suggesting that the AT1 receptor is not involved. Although [Sar'-Thr8]-AngII effectively antagonizes the actions of Ang-(1-7), its nonselectivity to the Ang receptor impedes the characterization of a specific role of Ang-(1-7) in blood pressure regulation. To overcome this problem, the antihypertensive effects of this peptide were assessed successfully using alternate strategies that depend on either the neutralization of the endogenous activity or inhibition of endogenous synthesis of Ang-(1-7).

In [mRen-2]27 transgenic hypertensive rats, cerebroventricular administration of a specific polyclonal Ang-(1-7) antibody resulted in a dose-dependent increase in blood pressure (62). Similarly, systemic administration of a monoclonal Ang-(1-7) antibody reversed the antihypertensive response produced in SHR after 8 days of treatment with lisinopril and losartan (Figure 2) (57). Administration of [Sar'-Thr8]-AngII also induced a pressor response in the SHR that was not prevented by prior blockade of either AT1 or AT2 receptor (57). On the other hand, prior administration of the monoclonal Ang-(1-7) antibody completely abolished the [Sar'-Thr8]-AngII-induced pressor response (Figure 3). These findings suggest that the vasodepressor action of ACE inhibition is mediated by Ang-(1-7) and that these effects are mediated via a non-AT1/AT2 receptor. Using a related strategy, inhibition of the endogenous synthesis of Ang-(1-7) by administering a specific neprilysin inhibitor also reversed the antihypertensive effect produced by the combined chronic administration of lisinopril and losartan in SHR (53). This approach resulted in a 60% decrease in the plasma concentrations of Ang-(1-7), which adds further support to the role of Ang-(1-7) in mediating the antihypertensive effects of ACE inhibition and the participation of neprilysin in the formation of Ang-(1-7).

**Renal Actions of Angiotensin-(1-7)**

The kidney plays an important role in maintaining body fluid and electrolyte balance and long-term blood pressure homeostasis through its unique structural and functional properties (63). The observation that angiotensin fragments possess bioactivity prompted studies of the effects of Ang-(1-7) in the regulation of renal function. The kidney may be a primary site for the synthesis of Ang-(1-7) (our unpublished observations) (64,65). In humans, the concentration of Ang-(1-7) in the renal vein is several times higher than in the blood (64). In pigs, the
Figure 2. Time course for the changes in mean arterial pressure (MAP) during a 15-min infusion of a specific monoclonal antibody to angiotensin-(1-7) in conscious spontaneously hypertensive rats 8 days after continuous therapy with a combination of lisinopril and losartan. Infusion of a comparable amount of purified mouse IgG had no effect on blood pressure. Values are means ± SEM. Reprinted with kind permission of the American Heart Association (from reference 57).

Figure 3. The hypertensive response produced by the intravenous infusion of an angiotensin-(1-7) monoclonal antibody is not modified by the consecutive administration of a competitive, nonselective AngII antagonist [Sar¹-Thr⁸]-AngII. Values are means ± SEM of the changes in MAP (●) and heart rate (HR; ○) in conscious spontaneously hypertensive rats after chronic therapy with lisinopril and losartan.

renal content of Ang-(1-7) is as high as AngI, whereas AngII is present only in small amounts (65).

Ang-(1-7) displays potent diuretic and natriuretic effects (23,25,26,66), although in water-loaded rats it caused antidiuresis through stimulation of vasopressin (67). Salt depletion causes a fourfold increase in urinary excretion of Ang-(1-7). In fact, this maneuver has a synergistic effect with ACE inhibitors and AngII antagonists and augments their antihypertensive
effects (68). These findings raise the question of whether increased formation of Ang-(1-7) during conditions of salt depletion is a mechanism that poses a limit to the antidiuretic and antinatriuretic actions of AngII. Additional work will be necessary to determine the modulatory actions of Ang-(1-7) on renal excretory capacity.

Several studies suggest that Ang-(1-7) functions as a paracrine hormone in the control of salt and water balance (17,23,25,69,70). Hilchey and Bell-Quilley (27) showed that Ang-(1-7) induces the intrarenal release of prostaglandins. These observations are consistent with those reported previously by our laboratory, which also showed that Ang-(1-7) stimulates prostaglandin release from a variety of other cells (29,71). The natriuretic effects of Ang-(1-7) are associated with increases in glomerular filtration rate and release of natriuretic prostaglandins, particularly PGL2 (26). Inhibition of PGL2 release with indomethacin was associated with a decrease in the natriuretic and diuretic actions of Ang-(1-7), supporting a role for the formation of PGL2 in mediating the renal actions of Ang-(1-7).

Studies in rats showed that Ang-(1-7) is excreted in urine at concentrations much higher than those determined for AnglI (72,73). In human subjects, Ang-(1-7) is excreted in the urine of normal healthy volunteers in amounts 2.5-fold higher than that measured in the plasma (61). However, concurrent studies in untreated essential hypertensive subjects showed a significant reduction in excretion of Ang-(1-7). In these studies, an inverse correlation was observed between urinary concentrations of Ang-(1-7) and blood pressure \( (r = 0.48, P < 0.001) \). Moreover, urinary Ang-(1-7) (odds ratio 0.92; 95% CI, 0.88 to 0.97) and age were independent predictors of the elevation in systolic blood pressure (61). Thus, the relatively higher concentrations of Ang-(1-7) in urine compared with plasma further support the data that suggest that locally produced Ang-(1-7) may contribute to the regulation of renal function.

Summary

Ang fragments possess biological activity, although their role in the maintenance of physiologic processes requires further investigation. Among angiotensin fragments, Ang-(1-7) is the most pleiotropic metabolite because it exerts effects that may be identical to or opposite those of AngII. Endogenous neutralization of the synthesis or activity of Ang-(1-7) revealed the involvement of this heptapeptide in the mechanism of action of ACE. These new studies expand our understanding of the mechanisms that may contribute to the antihypertensive effect of inhibition of ACE, particularly in view of the observation that neither inhibition of AngII synthesis nor accumulation of tissue bradykinin has sufficed to explain their mechanism of action. The demonstration that ACE metabolizes Ang-(1-7) suggests an alternate mechanism for the regulation of plasma or even tissue levels of Ang-(1-7), whereas the observation that the heptapeptide is a natural endogenous inhibitor of the C-domain of the enzyme provides another site at which changes in the activity of ACE may have important effects on the regulation of blood pressure. The studies reported above thus provide a new understanding of the contribution of the RAS in the long-term regulation of arterial pressure.

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