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## Regulation of Cardiovascular Control Mechanisms by Angiotensin-(1-7) and Angiotensin-Converting Enzyme 2

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### 1. INTRODUCTION

Among the molecular forms of angiotensin peptides generated by the action of renin on angiotensinogen (Aogen), both angiotensin II (Ang II) and the amino terminal heptapeptide angiotensin-(1-7) [Ang-(1-7)] are critically involved in the long-term control of tissue perfusion, cell-cell communication, development, and growth. Whereas an impressive body of literature continues to uncover pleiotropic effects of Ang II in the regulation of cell function, research on Ang-(1-7) has a shorter history as it was only 16 yr ago that a biological function for this heptapeptide was first demonstrated in the isolated rat neuro-hypophysial explant preparation (1). On the contrary, the synthesis of angiotonin/hypertensin (now Ang II) was first obtained in 1957 (2), three decades ahead of the discovery of Ang-(1-7) biological properties.

As Ang-(1-7) research continues to provide important and new information on the complexity of actions of the renin-angiotensin system (RAS) in homeostasis, it should be noted that one of the fundamental lessons learned from this discovery is that it

showed the existence of a feedback control mechanism whereby within the RAS a product, Ang-(1-7), regulates in an opposite manner the actions of the other product (Ang II) originating from a common substrate (angiotensin I) (3,4). The current status of our knowledge about the physiological actions of Ang-(1-7), its formation and degradation, and its role in cardiovascular disease are discussed in this chapter.

## 2. ANG-(1-7)-FORMING AND -DEGRADING ENZYMES

The major bioactive components of the RAS are produced from the conversion of angiotensinogen to the decapeptide angiotensin I (Ang I) in both the circulation and tissues. At these sites, the reaction of Ang I with angiotensin-converting enzyme (ACE) forms Ang II, whereas Ang-(1-7) is generated from Ang I by the action of endopeptidases. Generation of the two peptides possessing different carboxy termini and contrasting biological actions diverges through enzymic reactions from which ACE functioning as a di-peptidyl carboxypeptidase cleaves the Phe<sup>8</sup>-His<sup>9</sup> bond of Ang I to generate the octapeptide Ang II [Ang-(1-8)], whereas neutral endopeptidase 24.11 (neprilysin), 24.15 (thimet oligopeptidase), and 24.26 (prolyl-endopeptidase) cleave a tripeptide (Phe<sup>8</sup>-His<sup>9</sup>-Leu<sup>10</sup>) from Ang I to produce Ang-(1-7) (Fig. 1). Both ACE and the endopeptidases have a wide tissue distribution, but our studies suggest that the conversion of Ang I to Ang-(1-7) by the various peptidases may be determined by the relative abundance of the enzyme in the tissue or in the circulation. In accord with this interpretation, we showed that neprilysin forms Ang-(1-7) from Ang I in the circulation, prolyl-endopeptidase 24.26 may be more active in brain tissue (5) and in vascular endothelium (6) whereas thimet oligopeptidase 24.15 is an Ang-(1-7)-forming enzyme in vascular smooth muscle (7). The diversity of the Ang-(1-7)-forming enzymes reinforces our proposal that Ang-(1-7) may be a true *paracrine* or even *intracrine* hormone because its formation from Ang I could be determined in part by the relative abundance of the specific enzyme in either the circulation or the tissue compartment in which the Ang I substrate can react with the enzyme. The idea that tissue localization of the enzyme and its relative abundance are important is suggested by the demonstration that in renal tissue neprilysin degrades Ang-(1-7) into Ang-(1-4) (8,9).

Additional studies of the catabolic pathways for Ang-(1-7) degradation showed that ACE hydrolyzes the heptapeptide into Ang-(1-5) (10), a finding that signifies the contribution of the vasodilator and antiproliferative actions of Ang-(1-7) in explaining the mode of action of ACE inhibitors (11-14).

Angiotensin-converting enzyme 2 (ACE2) is a newly identified enzyme of the RAS that catalyzes the conversion of Ang I to Ang-(1-9) and, more importantly, converts Ang II into Ang-(1-7) (15-19) (Fig. 1). ACE2 exhibits a high catalytic efficiency for the latter reaction—almost 500-fold greater than that for the conversion of Ang I to Ang-(1-9). From an array of over 120 peptides, only dynorphin A and apelin 13 were hydrolyzed by ACE2 with comparable kinetics to the conversion of Ang II to Ang-(1-7) (18).

ACE2 was originally characterized as a homolog of ACE, sharing about 42% nucleotide sequence homology (19,20). Although both enzymes are type I glycoproteins, there are notable differences. The somatic form of ACE has two catalytic sites; on the other hand, both the testicular form of ACE and ACE2 have only one. ACE2 is a carboxymonopeptidase with a preference for hydrolysis between a proline and the



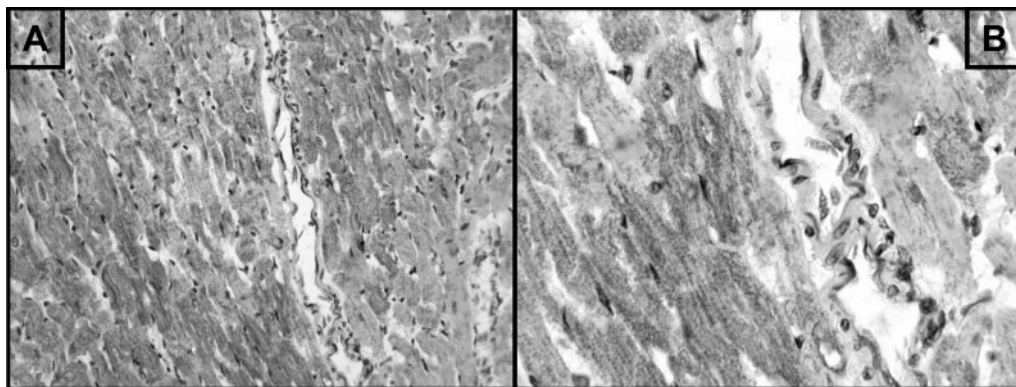


Fig. 2. Expression of Ang-(1-7)-like cardiac immunoreactivity in the rat. Ang-(1-7) staining was restricted to ventricular myocytes whereas it appeared as a granular reaction product throughout the cytoplasm (Panel A). The absence of Ang-(1-7) staining in endothelial and vascular smooth muscle cells of coronary vessels is best illustrated in the higher power magnification of Panel B. (See color version of this figure on color plates.)

complex. Early studies have demonstrated the Ang-(1-7) immunoreactivity in the cell bodies and in the axons of magnocellular neurons of the paraventricular nucleus (PVN) and supra-optic nucleus (SON) of the hypothalamus as well as in the neurons of the nucleus circularis (26). In fact, Ang-(1-7) immunoreactivity in neurons of the PVN and SON was co-localized with vasopressin-like immunoreactivity whereas Ang-(1-7) was not co-localized in paraventricular neurons immunoreactive for oxytocin (26,27). The same pattern of Ang-(1-7) immunoreactivity, seen in Sprague-Dawley rats, was also observed in the brains of (mRen2)27 transgenic rats (28) in which a subpopulation of nitric oxide synthase-containing neurons also contained Ang-(1-7)-like immunoreactivity. Collectively, the co-localization of Ang-(1-7) and vasopressin immunoreactivity in neurons of the magnocellular division of the PVN and the SON are congruent with functional studies demonstrating a role of Ang-(1-7) in the regulation of hydro-mineral balance involving neurons of the hypothalamic-neurohypophysial pathway. In addition, the co-localization of Ang-(1-7) immunoreactivity in nitric oxide synthase-containing neurons of the PVN is especially interesting because a number of studies now show that the angiotensin peptides may modulate the disposition of reactive oxygen species in the PVN of animals with heart failure (29).

Our interest in the role of Ang-(1-7) in cardiovascular regulation during heart failure led to the investigation of expression of Ang-(1-7) in the heart (30). Ang-(1-7) immunoreactivity in Lewis rats was restricted to myocytes of both the right and left ventricles. Fig. 2 shows that Ang-(1-7) staining in myocytes had a granular appearance throughout the cytoplasm. In contrast, there was a distinct absence of staining for the peptide in vascular smooth muscle as well as in interstitial cells of the heart. When Ang-(1-7) was examined in the hearts of rats subjected to ligation of the left main coronary artery, we observed a significant increase in Ang-(1-7) staining in ventricular myocytes that had undergone hypertrophic remodeling. In the region of ischemic damage there was a marked absence of Ang-(1-7) staining in fibroblasts and connective tissue. The increase in Ang-(1-7) staining in rats with congestive heart failure was positively correlated with an increase in left ventricular end-diastolic pressure and negatively correlated with

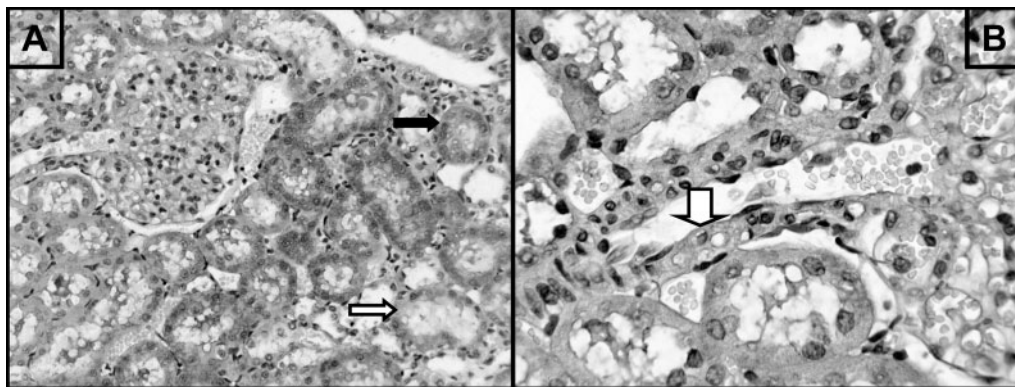


Fig. 3. Characteristics of Ang-(1-7)-like immunoreactivity in rat kidney. The most intense staining for Ang-(1-7) was in the thick-walled epithelial cells of the proximal-convoluted tubule (filled arrow in Panel A). A less intense staining for the peptide was observed in the thin-walled epithelial cells of collecting ducts (open arrow of Panel A). At both low (Panel A) and high (Panel B) power magnifications, there is essentially no staining for Ang-(1-7) in the glomerulus. Panel B also illustrates modest staining in the afferent arteriole (arrowhead in Panel B). (See color version of this figure on color plates.)

decreased cardiac contractility. These findings along with the report that Ang-(1-7) infusion in coronary ligated rats attenuated the development of heart failure (31) suggest that this peptide may play an important role in cardiac performance in cardiomyopathy.

Consistent with studies showing important actions of Ang-(1-7) in the regulation of renal function, characteristic features of Ang-(1-7) staining in the kidney are (1) varied intensity of staining in renal tubules with the degree of staining being more intense in proximal convoluted tubules and a lesser degree of staining in the thinner epithelial cells of the collecting ducts, (2) distinct absence of Ang-(1-7) immunoreactivity in the glomerulus, and (3) light staining in renal vessels (Fig. 3). Fig. 3B shows a granular reaction product for Ang-(1-7) staining in the juxtaglomerular cells of the afferent arteriole (32). It is interesting that blood vessels (arterioles) of the kidney exhibit Ang-(1-7) staining whereas this was not the observation for blood vessels in the heart or in other organs.

## 4. PHYSIOLOGICAL ACTIONS

### 4.1. Effects on the Vasculature

Vasodilation produced by Ang-(1-7) was first described in 1993 by Benter et al. (33) in rats that were made areflexic by spinal cord destruction. The vasodilator properties of the peptide were confirmed in isolated canine (34,35) and porcine (36) coronary artery vessels, rabbit pial arterioles (37), the rat mesenteric circulation (38), the spontaneously hypertensive rats (SHR) (39), [mRen2]27 hypertensive transgenic rats (40), two-kidney one-clip hypertensive dogs (41), and in the human forearm circulation (42). As reviewed elsewhere (43,44), the vasodilator response is mediated by release of vascular endothelium nitric oxide (NO), prostacyclin, and a receptor-mediated potentiation of bradykinin. The biological action of Ang-(1-7) satisfies the concept that the peptide acts to oppose the vasoconstrictor and hypertensive effects of Ang II by raising the activity of intrinsic vasodilator autocooids.

## 4.2. Effects on the Brain

Ang-(1-7) is present in hypothalamus, amygdala, and medulla oblongata at concentrations equivalent to or greater than those of Ang II (45) and evidence of a functional role for Ang-(1-7) exists in brain regions known to respond to other components of the RAS. Ang-(1-7) excites neurons in the PVN and in the nucleus of the solitary tract, dorsal motor nucleus of the vagus, and rostral and caudal ventrolateral medulla. The population of cells excited by Ang-(1-7) and Ang II shows some overlap, but the majority of cells respond to one or the other peptide. In addition to direct cellular actions, Ang-(1-7) releases other neurotransmitters including monoamines, substance P, vasopressin, glutamate, prostaglandins, and NO as reviewed recently (43). Like Ang II, the Ang-(1-7)-mediated release of vasopressin may involve tachykinin peptides (46-48). In contrast, intracerebroventricular injections of Ang-(1-7) do not increase blood pressure or promote drinking, which are thought to be mediated by Ang II acting on monoaminergic pathways (49,50). An opposing action of Ang-(1-7) on central mechanisms regulating blood pressure is demonstrated by the finding that cerebroventricular injections of antibodies to Ang-(1-7) produce opposite effects to those of Ang II (47). Evidence from studies using *c-fos* reveals that Ang-(1-7) activates pathways in the organum vasculosum of the lamina terminalis and median preoptic nucleus, whereas Ang II activates these pathways as well as those involving the PVN and subfornical organ (51,52). Pathways in the medulla oblongata appear separate for the two peptides as well, since sino-aortic denervation potentiated the responses to Ang-(1-7) but not those to Ang II in the nucleus of the solitary tract (53).

Important indicators of a role for the endogenous peptide in cardiovascular regulation come from studies using the Ang-(1-7) antagonist D-Ala<sup>7</sup>-Ang-(1-7) (44). Blockade of endogenous Ang-(1-7) within the solitary tract nucleus augments baroreceptor reflex control of heart rate, providing an independent and opposite effect from those of Ang II (54,55). A loss of tonic input by Ang-(1-7) may accompany the age-related decrease in reflex gain (56). In ASrAogen animals deficient of glial angiotensinogen, a role for endogenous Ang-(1-7) to facilitate baroreflex sensitivity persists, whereas the attenuating effect of Ang II is lost (57). These data suggest different sources of the two peptides consistent with their divergent functional effects. In the rostral and caudal ventrolateral medulla, Ang-(1-7) exerts excitatory actions mimicking those of Ang II (54,55), although clear differences exist in terms of regulation of the responses to the two peptides (58-60).

In brain, receptor subtypes exhibiting varying degrees of selectivity for either the AT<sub>1</sub> or AT<sub>2</sub> antagonists and D-Ala<sup>7</sup>-Ang-(1-7) appear to mediate the actions of Ang-(1-7) (43,61,62). The pharmacology of the receptor involved may be dependent on the specific transmitter studied, but blockade by D-Ala<sup>7</sup>-Ang-(1-7) is a common feature as reviewed in detail by Ferrario et al. (43). As an explanation for the fact that either D-Ala<sup>7</sup>-Ang-(1-7), AT<sub>1</sub> or AT<sub>2</sub> receptor antagonists, or some combination of the three antagonists is effective in blocking actions of Ang-(1-7) in brain, there is evidence that distinct subpopulations of classical AT<sub>1</sub> sites in the nucleus tractus solitarius (nTS) and dorsal motor nucleus of the vagus (dmnX) exist showing differential sensitivity/selectivity to Ang-(1-7) and the AT<sub>2</sub> antagonist (43). Moreover, AT<sub>1</sub> receptor antagonists (either losartan or candesartan) and D-Ala<sup>7</sup>-Ang-(1-7) block the actions of the peptide

in the nTS (63) and ventrolateral medulla (64). It is now recognized that the *mas* gene codes for a receptor responsible for vascular and renal effects of Ang-(1-7) (65). Mice deficient in the *mas* receptor show impairments in the baroreceptor reflex and alterations in responses to Ang II (64,66), providing additional evidence of a role for endogenous Ang-(1-7) in these functions.

### ***4.3. Effects on the Heart***

There are few studies about the direct actions of Ang-(1-7) on heart function, although the peptide is highly expressed in rat myocardium (30) and can be detected in larger concentrations in the cardiac interstitium or the coronary sinus blood after acute coronary artery ligation (67-69). In the isolated perfused heart of a rat, Ang-(1-7) appears to act as an anti-arrhythmogenic factor during reperfusion injury (70,71), although a study from the same group showed that Ang-(1-7) increased reperfusion arrhythmias (72). Functional data in whole animals suggest that Ang-(1-7) may have cardioprotective functions because an 8 wk infusion of Ang-(1-7) in Sprague-Dawley rats, started 2-wk postmyocardial infarction, attenuated heart failure progression together with restoration of vascular endothelial function (31). That the heart may be an important site for Ang-(1-7) actions is highlighted by the demonstration that ACE activity in plasma and atrial tissue is inhibited by Ang-(1-7) (73) whereas the peptide enhances tritiated norepinephrine release from isolated atrial tissue at doses comparable to those for Ang II (74).

The studies by us (69,75) and others on the relation between Ang-(1-7) function and cardiac ACE2 expression are shedding light on an intracardiac role for Ang-(1-7) as counterbalancing the hypertrophic and inotropic actions of Ang-(1-7) following myocardial injury. Ang-(1-7) was formed in the intact human myocardial circulation in patients with postcardiac transplantation and its levels were decreased when Ang II formation was suppressed by enalaprilat (76). The evolution of myocardial infarction in the rat 28 d after coronary artery ligation was accompanied by large increases in plasma Ang-(1-7) concentrations; further plasma Ang-(1-7) augmentation accompanied by reversal of cardiac remodeling because of continuous blockade of Ang II receptors with either losartan or olmesartan was accompanied by a threefold increase in cardiac ACE2 mRNA (75). In addition, emerging data suggest that Ang-(1-7) may oppose the atherogenic actions of Ang II through inhibition of smooth muscle proliferation and blockade of inflammatory cytokines. This interpretation is supported by the finding that the chronic effects of losartan in the prevention of fatty streak formation and monocyte activation in monkeys was associated with large increases in plasma Ang-(1-7) concentrations (77-81).

### ***4.4. Effects on the Kidney***

Although Ang-(1-7) is processed from either Ang I or Ang II in the circulation and in many tissues, it is important to emphasize that the processing pathways for Ang-(1-7) in the circulation and kidney are distinct. The endopeptidase neprilysin is the primary enzyme forming Ang-(1-7) from Ang I or Ang-(1-9) in the circulation (43,82). Although levels of neprilysin are low to undetectable, the enzyme is appropriately localized to the extracellular surface of endothelial and smooth muscle cells to contribute to the formation

of Ang-(1-7). In the kidney, Ang-(1-7) is the primary product formed in preparations of isolated proximal tubules and exists in urine at significantly higher levels than Ang II (8). Nephilysin may contribute to both the formation as well as the degradation of the peptide. Nephilysin cleaves Ang I to Ang-(1-7), but continues to metabolize Ang-(1-7) at the Tyr<sup>5</sup>-Ile<sup>6</sup> bond to form Ang-(1-4) and Ang-(5-7) (8,9). Indeed, nephilysin inhibitors increase the urinary levels of Ang-(1-7) in both human and rat (83,84). Moreover, the combined ACE/nephilysin inhibitor omapatrilat augmented renal and urinary levels of Ang-(1-7), but produced a blunted Ang-(1-7) response in plasma in comparison to ACE inhibition alone (32,84). Omapatrilat was also associated with increased expression of ACE2 and Ang-(1-7) in the proximal tubule (85). These data suggest that upregulation of ACE2 may contribute to the renal protective effects of ACE or combined vaso-peptidase inhibitors through increased conversion of Ang II to Ang-(1-7), as well as the reduced metabolism of Ang-(1-7). Indeed, ACE2 (-/-) mice exhibit enhanced intrarenal levels of Ang II and pronounced glomerulosclerosis (83).

We found that Ang-(1-7) and ACE2 are present within the proximal tubular regions of the mouse and the rat kidney (86,87). These data clearly support the concept of a complete RAS within the proximal tubule including expression of renin, ACE, angiotensinogen, the AT<sub>1</sub> and AT<sub>2</sub> receptors (88,89). The presence of Ang II and Ang-(1-7) in kidney supports the concept of important and divergent actions for the two peptides. For Ang II, renal actions include potent vasoconstriction, retention of sodium and water, as well as a stimulus for inflammation, and oxidative stress. In contrast, Ang-(1-7) stimulates diuresis and natriuresis that are associated with modest increases in the glomerular filtration rate (43). Ang-(1-7) induces vasodilation of afferent arterioles through a NO-dependent pathway (90). Indeed, immunocytochemical staining for the Ang-(1-7) receptor *mas* is evident in the afferent arteriole, as well as throughout the proximal tubules of the renal cortex providing biochemical support for the functional actions of the peptide (87). Ang-(1-7) and its metabolite Ang-(3-7) are potent inhibitors of Na<sup>+</sup>, K<sup>+</sup>-ATPase activity in the renal epithelium (91-93). Ang-(1-7) also inhibits the transcellular flux of sodium, which was associated with activation of phospholipase A<sub>2</sub> (PLA<sub>2</sub>) (94). Ang-(1-7) dependent inhibition of sodium transport is potentiated by ACE inhibition suggesting a shift toward the formation and protection of Ang-(1-7) within the proximal tubules. The chronic and pronounced diuresis following omapatrilat treatment was associated with large increases in urinary excretion of Ang-(1-7) and enhanced immunocytochemical staining of the peptide in the kidney (32). Recent studies have revealed that Ang-(1-7) abolished the Ang II-dependent stimulation of the Na<sup>+</sup>-ATPase activity in ovine kidney (95,96). Furthermore, intrarenal administration of Ang-(1-7) blocked the antinatriuretic actions of Ang II (97). These studies emphasize that the actions of Ang-(1-7) within the kidney may be particularly relevant in the setting of an activated RAS. Moreover, the discrete localization of Ang-(1-7), ACE2, and the *mas* receptor provides evidence for an alternative RAS within the proximal tubule epithelium that may antagonize the actions of Ang II in this renal compartment.

#### **4.5. Ang-(1-7) in Gestation and Pregnancy**

Pregnancy is a physiological condition characterized by increased RAS activity (98) that does not manifest in increased blood pressure (99). Merrill et al. (100) evaluated the effect of pregnancy on Ang-(1-7) in nulliparous preeclamptic patients and in third trimester



normotensive pregnant controls (3rd T; matched for parity, race, and gestational age). Plasma Ang-(1-7) was increased by 34% ( $p < 0.05$ ), whereas plasma Ang II was increased by 50% ( $p < 0.05$ ). In preeclampsia subjects plasma Ang-(1-7) was reduced ( $13 \pm 2$  pg/mL,  $p < 0.05$  vs third T); plasma Ang II was also reduced ( $32 \pm 4$  pg/mL, preeclamptic vs 3rd trimester normal pregnant,  $p < 0.05$ ), but remained elevated as compared to nonpregnant subjects and 50% higher than plasma Ang-(1-7). Other components of the RAS, with the exception of ACE, were reduced in preeclamptic subjects. Assessment of the relationship between Ang-(1-7) and blood pressure revealed a negative correlation of Ang-(1-7) with systolic ( $r = -0.4$ ,  $p < 0.02$ ) and diastolic ( $r = -0.5$ ,  $p < 0.02$ ) blood pressures. These data suggested a potential role for reduced production of Ang-(1-7) contributing to the elevated blood pressure. In preeclampsia, the decreased levels of plasma Ang-(1-7) in the presence of persistent elevated plasma Ang II are consistent with the development of hypertension.

Additionally, a 24-h urinary excretion of Ang-(1-7) and Ang II was evaluated during the ovulatory menstrual cycle, single normotensive pregnancies, and their subsequent lactation (101). No significant differences in urinary Ang-(1-7) were observed between the follicular and luteal phase of the normal menstrual cycle. There was a progressive rise of urinary Ang-(1-7) throughout normal human gestation, attaining levels that are 10-fold greater than that of the normal menstrual cycle. Urinary Ang II showed a similar pattern reaching levels that were 25-fold higher than the values at the menstrual cycle. At 35 wk of gestation, Ang-(1-7) was the predominant angiotensin peptide in the urine, reaching levels that were sixfold higher than Ang II. The urinary excretion levels may reflect local kidney production of peptides. Thus, increases in renal Ang-(1-7) levels may play a role in the vasodilatory adaptations of mid and late human pregnancies.

To understand the contribution of the RAS during pregnancy, studies were conducted in pregnant rats at late gestation (19th day) and compared to virgin female rats at diestrus phase of the estrous cycle. Twenty-four hour urinary excretion of the angiotensin peptides was significantly increased in pregnant animals by 93% (Ang I), 44% (Ang II), and 60% [Ang-(1-7)] of values found in virgin rats. Kidney Ang I and Ang-(1-7) concentrations were significantly increased by seven- and fivefold, respectively ( $p < 0.05$ ) in pregnant animals as compared to virgin females. In contrast, there was no significant change in renal Ang II concentrations of pregnant and virgin females. These studies provide evidence that urinary excretion of angiotensin peptides reflect local kidney content of angiotensins during pregnancy. The potential contribution of Ang-(1-7) to vascular control in pregnancy was also documented from increased vasodilator responses to the local application of the peptide in isolated small mesenteric arteries obtained from pregnant rats (102). In alignment with this interpretation, Ang-(1-7) and ACE2 staining in the kidney of 19 d pregnant Sprague-Dawley rats showed higher intensity when compared with virgin rats (103).

## 5. ANG-(1-7) RECEPTOR MECHANISMS

Ang-(1-7) is a poor competitor at pharmacologically defined AT<sub>1</sub> or AT<sub>2</sub> receptors (104-107). Santos et al. (62) designed a selective antagonist for the Ang-(1-7) receptor by replacing the a-proline at position 7 of Ang-(1-7) with D-alanine [D-Ala<sup>7</sup>]-Ang-(1-7). In initial studies, [D-Ala<sup>7</sup>]-Ang-(1-7) blocked hemodynamic and renal effects of

Ang-(1–7), did not compete for binding at rat adrenal AT<sub>1</sub> or AT<sub>2</sub> receptors, and did not attenuate pressor or contractile responses to Ang II, demonstrating selectivity for Ang-(1–7). We identified Ang-(1–7) binding sites on bovine aortic endothelial cells (BAEC), canine coronary artery rings, and rat blood vessels that are sensitive to [D-Ala<sup>7</sup>]-Ang-(1–7) (108–110). In addition, a multitude of physiological responses to Ang-(1–7) are selectively blocked by [D-Ala<sup>7</sup>]-Ang-(1–7) or the sarcosine analogs of Ang II, but not by AT<sub>1</sub> or AT<sub>2</sub> receptor antagonists, including the depressor response to Ang-(1–7) in the pithed rat and the lowering of blood pressure in hypertensive rats (33,40,111). Collectively, these results demonstrate that the hypotensive response to Ang-(1–7) is mediated by a non-AT<sub>1</sub>, non-AT<sub>2</sub>, [D-Ala<sup>7</sup>]-Ang-(1–7)-sensitive receptor. We refer to this receptor as the AT<sub>(1–7)</sub> receptor, as defined by its sensitivity to Ang-(1–7), its antagonism by [Sar<sup>1</sup>-Thr<sup>8</sup>]-Ang II and [D-Ala<sup>7</sup>]-Ang-(1–7), and its lack of response to AT<sub>1</sub> or AT<sub>2</sub> receptor antagonists, either functionally or in competition for binding.

Identification of an AT<sub>(1–7)</sub> receptor is confounded by reports of responses to Ang-(1–7) that are sensitive to AT<sub>1</sub> or AT<sub>2</sub> receptor antagonists or both. Some of the renal and central effects of Ang-(1–7) are mediated by a losartan-sensitive receptor (64,91,112). Arachidonic acid release from rabbit VSMCs and hypothalamic norepinephrine release were blocked by both [D-Ala<sup>7</sup>]-Ang-(1–7) and the AT<sub>2</sub> antagonist PD123319 (113,114). Additional responses to Ang-(1–7) in brain and heart are blocked by both AT<sub>1</sub> and AT<sub>2</sub> receptor antagonists (74,115). These results provide evidence for additional subtypes of the AT<sub>(1–7)</sub> receptor that are sensitive to losartan and/or PD123319 or suggest an interaction with the AT<sub>1</sub> and/or the AT<sub>2</sub> receptor.

Many of the physiological and cellular responses that are mediated by the AT<sub>(1–7)</sub> receptor are linked to the production of prostaglandins. Ang-(1–7) stimulates prostaglandin production in endothelial cells, VSMCs, astrocytes, and renal tubular epithelial cells (74,94,104–107,115). In addition, physiological responses to Ang-(1–7) are dependent on prostanoid production, based on the studies using the cyclooxygenase inhibitor indomethacin (Fig. 4).

Recently, Santos et al. (65) reported that the orphan G protein-coupled receptor *mas* is an Ang-(1–7) receptor. Ang-(1–7) bound with high affinity to cells transfected with the *mas* receptor, which was blocked by [D-Ala<sup>7</sup>]-Ang-(1–7), and renal or depressor responses to Ang-(1–7) were lost in *mas*-depleted mice. We recently showed that antisense oligonucleotides or siRNAs to *mas* prevent the Ang-(1–7)-mediated inhibition of growth in VSMCs, which is also blocked by [D-Ala<sup>7</sup>]-Ang-(1–7) (116). These results suggest that the *mas* receptor serves as a selective Ang-(1–7) binding site. Fig. 4 shows the signal transduction pathway by which Ang-(1–7) activates the G protein-coupled *mas* receptor to increase the production of NO and prostacyclin (PGI<sub>2</sub>) via increases in cGMP and cAMP, respectively. Ang-(1–7) also reduces the mitogen-activated protein kinases (MAPKs) by either increasing MAPK phosphatases or reducing the MAPK kinase MEK. The increase in cAMP and cGMP and the decrease in MAPK activity cause vasodilation and inhibit cell growth. The *mas* receptor is predominantly expressed in the testis and the hippocampus and amygdala of the mammalian forebrain with minimal levels in the rodent heart and kidney. This tissue distribution differs from previous reports of Ang-(1–7) binding and functional responses, suggesting the existence of other AT<sub>(1–7)</sub> receptors.

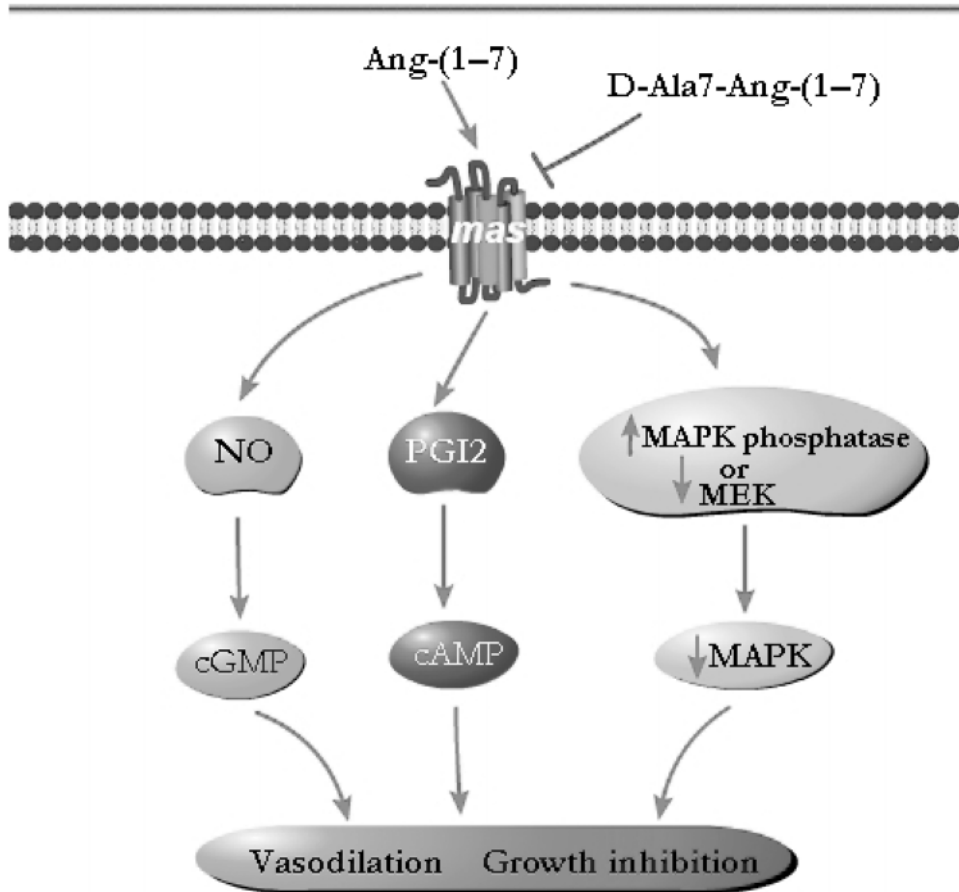


Fig. 4. The major signal transduction pathways for Ang-(1-7). Ang-(1-7) activates the G protein-coupled *mas* receptor to increase the production of nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>) to increase cGMP and cAMP, respectively. Ang-(1-7) also reduces the mitogen-activated protein kinases (MAPKs) by either increasing MAPK phosphatases or reducing the MAPK kinase MEK. The increase in cAMP and cGMP and the decrease in MAPK activity cause vasodilation and inhibit cell growth. (See color version of this figure on color plates.)

## 6. SUMMARY

Ang-(1-7), a product of both Ang I and Ang II metabolisms, functions to antagonize the actions of Ang II by acting primarily through binding to a non-AT<sub>1</sub>/AT<sub>2</sub> receptor and also the *mas* receptor. Ang-(1-7) stimulates vasodilation through increased production of vasodilator prostaglandins and NO, as well as amplifying the intrinsic actions of bradykinin. In the kidney, Ang-(1-7) promotes natriuresis and diuresis through an effect on transport mechanisms involved in water and electrolyte absorption within the renal tubules and collecting ducts. In addition, Ang-(1-7) modulates the actions of tubular vasopressin via an effect on its V2 receptor. In the heart, Ang-(1-7) counteracts the hypertrophic, pro-fibrotic, and pro-thrombotic effects of Ang II as well as increases myocardial blood flow.

Ang-(1-7) contributes to the antihypertensive effects of ACE inhibitors and Ang II antagonists by several mechanisms (84,117,118,119). Inhibition of ACE increases blood and tissue concentrations of Ang-(1-7) by preventing ACE-mediated Ang-(1-7) degradation and increasing substrate (Ang I) availability. In contrast, blockade of Ang II receptors increases blood and tissue levels of Ang-(1-7) by: (i) increasing Ang I substrate availability through the dis-inhibition of Ang II-mediated renin release, and (ii) augmenting the rate of Ang II conversion into Ang-(1-7) via increased ACE2 expression and activity. Both ACE and ACE2 represent critical steps at which modulation of angiotensin peptide functions are precisely regulated, the specific mechanisms of which need further investigation. Genomic studies are also urgently needed to determine whether polymorphisms in the ACE2 gene or the genes for Ang-(1-7)-forming enzymes exist.

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