# Predominance of $AT_1$ Blockade Over *Mas*–Mediated Angiotensin-(1–7) Mechanisms in the Regulation of Blood Pressure and Renin–Angiotensin System in mRen2.Lewis Rats

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**BACKGROUND**
We investigated whether the antihypertensive actions of the angiotensin II (Ang II) receptor ($AT_1$-R) blocker, olmesartan medoxomil, may in part be mediated by increased Ang-(1–7) in the absence of significant changes in plasma Ang II.

**METHODS**
mRen2.Lewis congenic hypertensive rats were administered either a vehicle (n = 14) or olmesartan (0.5 mg/kg/day; n = 14) by osmotic minipumps. Two weeks later, rats from both groups were further randomized to receive either the *mas* receptor antagonist A-779 (0.5 mg/kg/day; n = 7 per group) or its vehicle (n = 7 per group) for the next 4 weeks. Blood pressure was monitored by telemetry, and circulating and tissue components of the renin–angiotensin system (RAS) were measured at the completion of the experiments.

**RESULTS**
Antihypertensive effects of olmesartan were associated with an increase in plasma renin concentration, plasma Ang I, Ang II, and Ang-(1–7), whereas serum aldosterone levels and kidney Ang II content were reduced. Preserved Ang-(1–7) content in kidneys was associated with increases of ACE2 protein but not activity and no changes on serum and kidney ACE activity. There was no change in cardiac peptide levels after olmesartan treatment. The antihypertensive effects of olmesartan were not altered by concomitant administration of the Ang-(1–7) receptor antagonist except for a mild further increase in plasma renin concentration.

**CONCLUSIONS**
Our study highlights the independent regulation of RAS among plasma, heart, and kidney tissue in response to $AT_1$-R blockade. Ang-(1–7) through the *mas* receptor does not mediate long-term effects of olmesartan besides counterbalancing renin release in response to $AT_1$-R blockade.

*Keywords:* angiotensin II; angiotensin-(1–7); $AT_1$ receptor; blood pressure; heart; hypertension; kidney; mas receptor; olmesartan.

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Although the mechanism of action of angiotensin II (Ang II) receptor ($AT_1$-R) blockers (ARBs) is well established, data suggest that their antihypertensive effects may be limited in part by the overriding effect of compensatory increases in plasma renin activity (PRA) and Ang II.[1] Contrasting with the mechanism of action of other ARBs, Agata *et al.*[2] reported that the antihypertensive and cardiac antihypertrophic actions of 4-week olmesartan medoxomil administration were not associated with the expected increases in circulating Ang II in adult stroke-prone spontaneously hypertensive rats (SHR). Agata *et al.*[2] speculated that the failure of plasma Ang II to rise in response to blockade of $AT_1$-R could be explained by angiotensin-(1–7) [Ang-(1–7)]–induced inhibition of angiotensin converting enzyme (ACE) activity, given previous observations of increased Ang-(1–7) after treatment with ARBs,[3,4] as well as an inhibitory role of the heptapeptide at the C- and N-terminus catalytic activity of the enzyme.[5,6] The intriguing possibility of a differential effect of olmesartan on plasma Ang II levels compared with other ARBs was also consistent with 2 other studies in human essential hypertensive subjects in whom prolonged therapy with olmesartan was accompanied by either decreases or no changes in plasma Ang II levels.[7,8]

The effects of olmesartan on Ang II levels may also result from increased conversion of the octapeptide to Ang-(1–7) because ACE2 has been shown to be

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upregulated by $AT_1$-R blockade.[4,9,10] This has been demonstrated *in vivo* by recent work showing that the infusion of soluble human recombinant ACE2 efficiently lowered plasma Ang II while increasing Ang-(1–7).[10] Furthermore, in isolated cardiac myocytes, ACE2 messenger RNA expression and activity were not affected by Ang-(1–7); however, the inhibitory effects of Ang II on ACE2 were blocked by Ang-(1–7).[11] The heptapeptide modulatory effect was prevented by the Ang-(1–7) *mas* receptor antagonist [D-ALA$^7$]-Ang-(1–7) (A-779), indicating that the Ang-(1–7) response was mediated by a specific Ang-(1–7) receptor. A-779 is a selective blocker of the *mas* receptor that has been identified to mediate vasodilatory, antitrophic, and antiproliferative effects of Ang-(1–7).[12–14]

The long-term effects of Ang-(1–7) antagonism in the presence of concomitant Ang II receptor blockade have not been determined. With this in mind, we investigated the Ang-(1–7)–mediated effects of olmesartan on blood pressure, plasma, renal, and cardiac Ang II as well as ACE2 in mRen2.Lewis congenic hypertensive rats. This monogenetic hypertensive rat strain was developed in our laboratory through a backcross of the hypertensive (mRen2)27 transgenic rats with normotensive Lewis rats. The aim of this backcross was to offset the heterogeneity of the parent strain that contributed to the genetic variability found within the original transgenic strain.[15,16] Because the malignant phase of hypertension is not observed in mRen2.Lewis rats, the longer life span of this experimental model provides a better opportunity to investigate the function and regulation of tissue renin–angiotensin system (RAS) and its contribution to the etiology of hypertension and target organ damage.

## METHODS

### Experimental protocol

Twenty-eight hemizygous male mRen2.Lewis hypertensive rats were obtained from the congenic colony founded at the Wake Forest University Hypertension and Vascular Research Center. Rats were housed in an American Association of Laboratory Animal Care–approved facility in a temperature-controlled room (22 ± 2 °C) with a 12:12-hour light/dark cycle (lights on from 6:00 AM to 6:00 PM) and were allowed free access to food and water. The rats were handled in accordance with National Institute of Health guidelines; our Institutional Animal Care and Use Committee approved the study in advance. At age 10 weeks and under aseptic conditions, radiotelemetry probes (PA-C40; DSI, St. Paul, MN) were chronically implanted under anesthesia for continuous monitoring of arterial pressure and heart rate, as described elsewhere.[17] After a 2-week recovery period, animals were randomized to receive either vehicle (2.5% sodium bicarbonate; n = 14) or olmesartan (Daiichi Sankyo, Inc., Parsippany, NJ; 0.5 mg/kg/day dissolved in 2.5% sodium bicarbonate; n = 14) by osmotic minipumps implanted subcutaneously for the ensuing 2 weeks (Figure 1). Thereafter, rats from both groups were randomized to receive either the Ang-(1–7) antagonist A-779 (Bachem, Torrance, CA; 0.5 mg/kg/day in mili-Q water; n = 7) or its vehicle (mili-Q water; n = 7) for the next 4 weeks. Two-week pumps implanted initially at the beginning of the therapeutic period were replaced at the same time with new pumps to cover the remaining 4 weeks of the experiment. As shown in Figure 1, the design of the study allowed us to assess the effects of vehicle or olmesartan alone or in combination with A-779. After 6 weeks on the respective treatment, animals

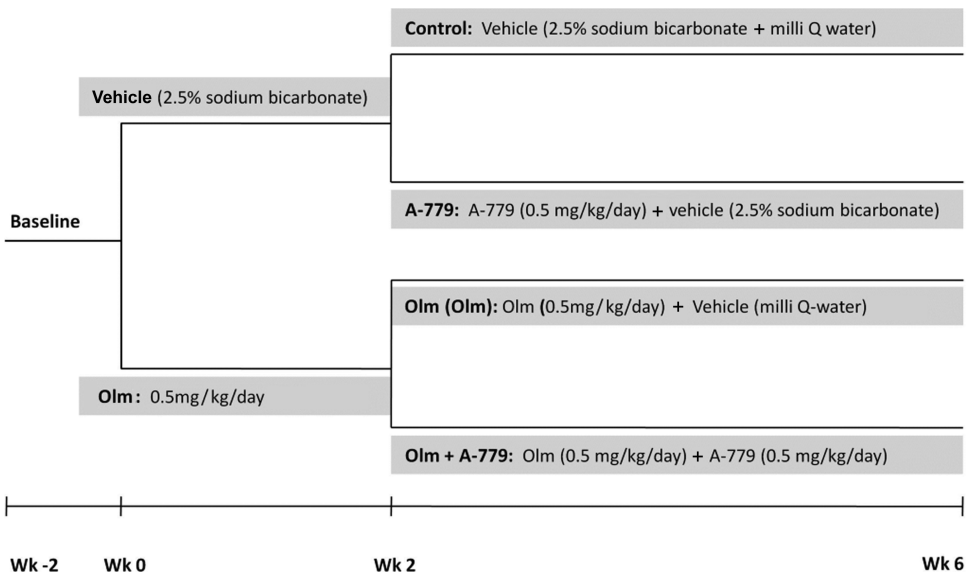


**Figure 1.** Outline of experimental design. Male mRen2.Lewis congenic hypertensive rats were implanted with radio-telemetry probes at age 10 weeks. After 2-week recovery period, rats were randomized to receive either vehicle (2.5% sodium bicarbonate; n = 14) or olmesartan (Olm; 0.5 mg/kg/day dissolved in 2.5% sodium bicarbonate; n = 14) by osmotic minipumps implanted subcutaneously for the ensuing 2 weeks. After the 2-week period on the respective treatment, rats from both groups were randomized to receive either the *mas* receptor antagonist A-779 (0.5 mg/kg/day in mili Q water; n = 7) or its vehicle (mili Q water; n = 7) for the next 4 weeks. Two-week pumps implanted initially at the beginning of the therapeutic period were replaced at the same time with new pumps to cover the remaining 4 weeks of the experiment.

were decapitated, and trunk blood was collected for measurements of renin–angiotensin–aldosterone system components. In addition, their heart and kidneys were removed and weighed, and the tissue samples were quickly frozen on dry ice for later measurement of angiotensin peptides and ACE, ACE2, and neprilysin (NEP) activities.

### Plasma and tissue hormone assays

Plasma renin concentration (PRC) and plasma and tissue Ang I, Ang II, and Ang-(1–7) were measured by radioimmunoassays, as previously described (details provided in Supplementary Methods).[3]

### Enzyme assays

Serum and tissue ACE activity was determined with the synthetic substrate Hip-His-Leu.[18] Tissue ACE2 and NEP activity were measured by high-performance liquid chromatography (HPLC), as previously described (details provided in Supplementary Methods).[3]

### Western blotting

ACE2 protein was analyzed by Western blot, as previously described (details provided in Supplementary Methods).[3]

### Statistics

All values are expressed as the mean ± 1 SEM. Data were analyzed by use of analysis of variance followed by Newman–Keuls posttest. A value of $P < 0.05$ was considered to be of statistical significance.

## RESULTS

### Body and organs weight

Body and kidney weights were not different between experimental groups, whereas heart weight and the heart weight–to–body weight ratios were significantly lower in both olmesartan and olmesartan plus A-779 groups when compared with the control and A-779–treated animals (Table 1).

### Blood pressure and heart rate

Mean blood pressure was lower in rats treated with olmesartan throughout the whole experimental period, whereas addition of A-779 had no effect on either the mean blood pressure or the heart rate of rats medicated with olmesartan or the vehicle (Figure 2). Heart rate was not different between the groups except for the first few days after olmesartan administration when higher heart rate followed the profound reduction in blood pressure in groups treated with the $AT_1$-R antagonist (Figure 2).

### Plasma and tissue RAS

PRC and plasma concentrations of Ang I, Ang II, and Ang-(1–7) were significantly higher in olmesartan and olmesartan plus A-779 groups compared with the control and A-779 groups (Figure 3a). Concomitant treatment with A-779 further raised PRC in olmesartan-treated rats. Serum levels of aldosterone were lower in both olmesartan-treated groups, although they reached the level of statistical significance only when compared with A-779. Serum ACE activity was not different among the experimental groups (Figure 3a). Neither olmesartan nor A-779 treatment changed angiotensin peptide concentrations in heart tissue (Figure 3b). In contrast, renal cortical Ang I increased significantly whereas Ang II decreased in both olmesartan and olmesartan plus A-779 groups when compared with the control and A-779 groups. There was no difference in Ang-(1–7) concentration in kidney cortex among the experimental groups.

To further elucidate the effects of $AT_1$ and *mas* receptor antagonism on renal angiotensin peptide levels we measured in kidney cortex membranes protein expression and activity of ACE2, the enzyme that preferentially cleaves Ang II into Ang-(1–7).[19] ACE2 protein expression, but not enzymatic activity, was increased significantly in both groups treated with olmesartan, whereas A-779 had no effect on ACE2 in both control and olmesartan-treated groups (Figure 4 and Table 2). In addition, neither treatment significantly affected ACE or NEP activities (Table 2).

## DISCUSSION

The results from this study add additional weight to the concept of independent regulation of angiotensin peptides content among plasma, heart, and kidney tissue in response to $AT_1$-R blockade. In addition, we showed that the antihypertensive effects of olmesartan treatment are associated with marked suppression of serum aldosterone levels despite the presence of elevated plasma Ang I and Ang II levels. The reactive hyperreninemia associated with the antihypertensive

**Table 1.** Effect of treatments on body, kidney, and heart weights in congenic mRen2.Lewis rats

| Variables | Control | A-779 | OLM | OLM + A-779 |
|---|---|---|---|---|
| Body weight, g | 436 ± 7 | 447 ± 5 | 436 ± 9 | 442 ± 11 |
| Left kidney weight, g | 1.46 ± 0.01 | 1.52 ± 0.03 | 1.48 ± 0.02 | 1.48 ± 0.05 |
| Right kidney weight, g | 1.53 ± 0.02 | 1.56 ± 0.03 | 1.53 ± 0.02 | 1.53 ± 0.04 |
| Heart weight, g | 1.35 ± 0.02 | 1.41 ± 0.02 | 1.03 ± 0.04* | 1.01 ± 0.02*,‡ |
| Heart weight/body weight ratio, mg/kg | 3.09 ± 0.05 | 3.16 ± 0.04 | 2.37 ± 0.10* | 2.29 ± 0.05*,‡ |

Values are means ± SEM. A-779, *mas* receptor antagonist [D-ALA$^7$]-Ang-(1–7).
Abbreviation: OLM, olmesartan.
*$P < 0.05$ vs. control; ‡$P < 0.05$ vs. A-779.

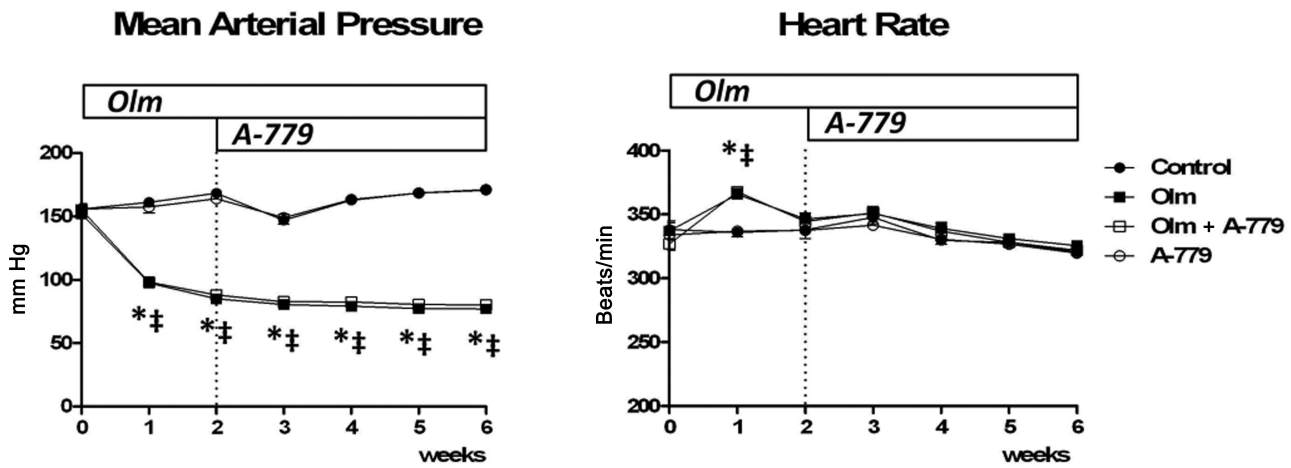


**Figure 2.** Weekly arterial blood pressure and heart rate profiling by telemetry in congenic mRen2.Lewis rats. Olmesartan (Olm) reduced blood pressure in congenic rats from the first week of the treatment ($P < 0.05$ vs. control and *mas* receptor antagonist [D-ALA$^7$]-Ang-(1–7) (A-779)). Coadministration of A-779 did not affect the blood pressure–lowering effects of olmesartan nor did the treatment with A-779 alone induce any change in blood pressure. The profound antihypertensive effect of olmesartan was associated with an increase in heart rate in olmesartan and olmesartan + A-779 ($P < 0.05$ vs. control and A-779) that returned to the pretreatment values after the first week of treatment. Values are mean ± SEM. n = 7 per group. *$P < 0.0.5$ vs. control; ‡$P < 0.0.5$ vs. A-779.

actions of olmesartan treatment and consequent $AT_1$-R blockade induced differential changes in the expression of Ang I and Ang II content in heart and renal cortical tissues that were characterized by prominent and opposing changes in renal Ang I and Ang II, respectively, and no changes in their content in heart tissue. Reduced $AT_1$-R–mediated uptake seems to predominantly contribute to decreased renal Ang II content, although the 2-fold increases in the ACE2 protein suggest an additional contribution through increased Ang II metabolism. However, we could not demonstrate an increase in ACE2 enzymatic activity despite increase in protein levels in this study. Although the $AT_1$-R blockade increased plasma Ang-(1–7) levels and preserved the renal content of the heptapeptide, the absence of the effects of A-799 suggests that the *mas* receptor does not mediate long-term effects of olmesartan on blood pressure or profile of angiotensin peptides in congenic mRen2.Lewis rats. The augmentation in PRC due to the combined effect of both $AT_1$ and *mas* receptor blockade is a new finding implicating a counterbalancing role for Ang-(1–7) in the control of renin release.

Telemetric measurements of arterial pressure, by providing high precision and accuracy, showed that *mas* receptor blockade had no effect on the 24-hour blood pressure of either vehicle- or olmesartan-treated rats. The absence of an effect of A-779 on blood pressure cannot be due to insufficient blockade because the doses used in these experiments are within the range reported by others[10,20,21] and the same as those used by Agata *et al.*[2] As first pointed out by Ferrario,[22] the direct effects of Ang-(1–7) on blood pressure can be demonstrated only in conditions in which compensatory mechanisms are rendered ineffective or in the presence of significant RAS overactivity. Although direct antihypertensive effects of Ang-(1–7) administration have been documented in SHR[23] and SHR treated with NG-nitro-L-arginine methyl ester (L-NAME)[24], a chronic infusion of Ang-(1–7) did not modify the course of 2K-1C hypertension.[25] Although A-779 has been reported to reverse the blood pressure–lowering effects of Ang-(1–7) or an ACE inhibitor in an experimental model of diabetes,[24,26] neither stimulation nor blockade of the *mas* receptor influences blood pressure in WKY–[23] or Ang II–infused Sprague–Dawley rats.[27] Moreover, in mice receiving an acute infusion of human recombinant ACE2 and Ang II, neither the infusion of Ang-(1–7) nor the infusion of a *mas* receptor blocker had any effect on blood pressure.[10] Our study further shows in a chronic setting that blockade of $AT_1$-R–dependent changes in blood pressure predominates over the potential impact of additional *mas*-R–mediated vasodilator mechanisms related to the observed increase in Ang-(1–7) levels. This conclusion does not negate a contribution of *mas* receptor–mediated Ang-(1–7) actions in conditions of reduced circulating Ang II, such as those resulting from ACE inhibition, because we showed that the blood pressure increase in response to acute Ang-(1–7) neutralization was larger in lisinopril- vs. losartan-treated SHR.[28] In addition, in the presence of prolonged $AT_1$-R blockade, circulating Ang II may increase to an extent that the peptide interacts with a D-[Ala$^7$]-Ang-(1–7)–sensitive site.[29] It is possible that in that way Ang II prevents the effects of Ang-(1–7) on *mas* receptor without any particular effects of its own. Moreover, previous studies also showed that Ang-(1–7) effects are mediated through receptors other then *mas*, for example $AT_2$.[20] Finally, Ang-(1–7), like olmesartan,[1,30,31] may elicit inverse agonist effect on $AT_1$-Rs. Thus, Ang-(1–7) and olmesartan may have overlapping activities at the $AT_1$-Rs and that may be why A-779 has no apparent blood pressure effect when given together with olmesartan. Further experiments are necessary to confirm the attractive hypothesis that $AT_1$-Rs must be unblocked for Ang-(1–7) to show an antihypertensive potential.

In 2006, Ichikawa and Takayama[7] reported in hypertensive patients that olmesartan either did not change or decreased circulating Ang II in association with decreases in serum aldosterone after longer-term administration (2 years). Those findings were in sharp contrast with the

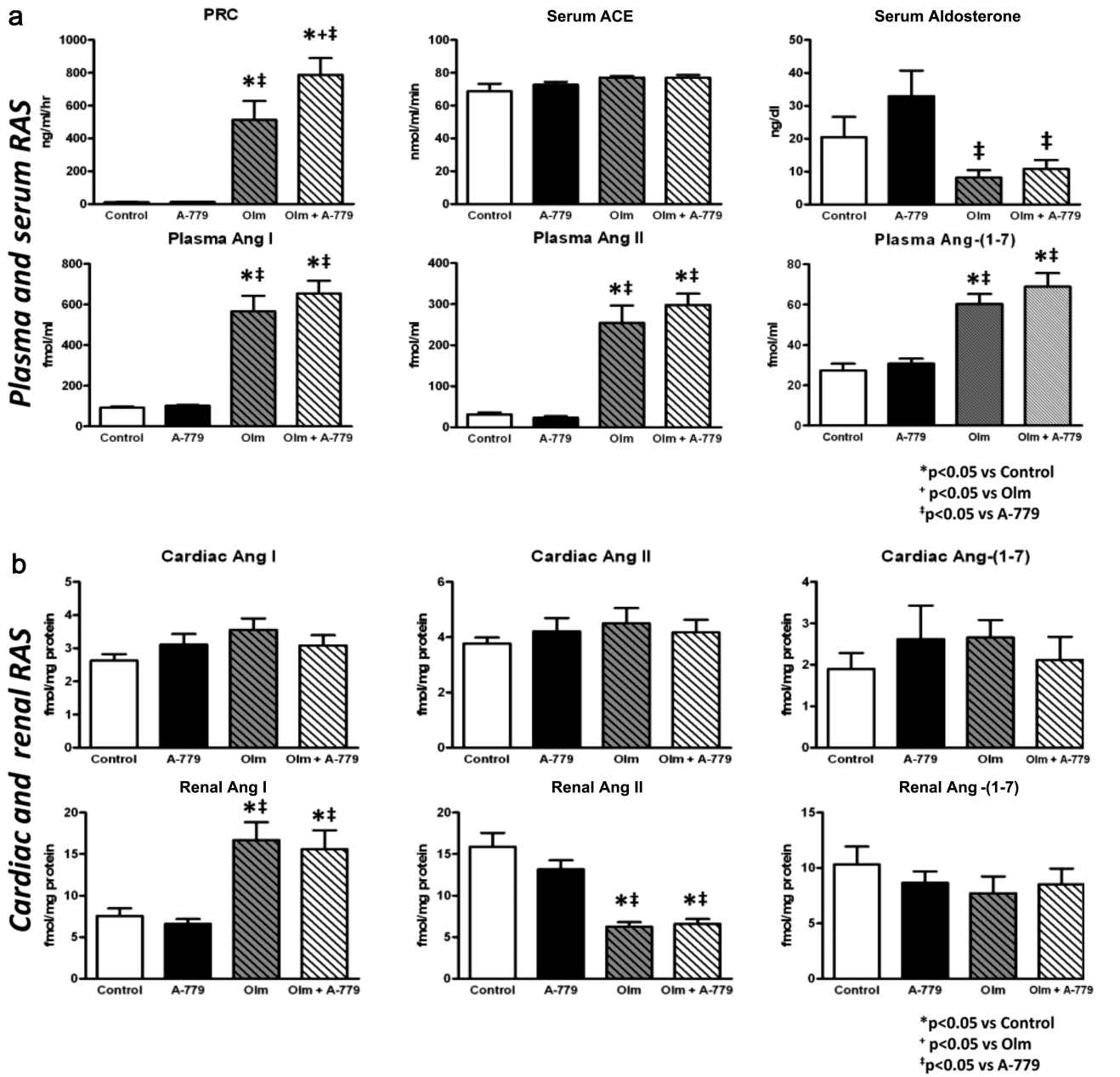


**Figure 3.** Effects of $AT_1$ and *mas* receptor antagonists on circulating (**a**), heart (**b**), and kidney cortex (**b**) renin–angiotensin–aldosterone system components. Values are expressed as mean ± SEM. n=7 per group. Abbreviations: Ang I, angiotensin I; Ang II, angiotensin II; Ang-(1–7), angiotensin-(1–7); A-779, *mas* receptor antagonist [D-ALA$^7$]-Ang-(1–7); Olm, olmesartan; RAS, renin–angiotensin system. *$P < 0.05$ vs. control; +$P < 0.05$ vs. Olm; ‡$P < 0.05$ vs. A-779.

effects of other $AT_1$-R antagonists that largely increase PRC and consequently plasma Ang II. Comparative findings reported by Tsutamoto *et al.*[8] showed that a 12-month exposure to olmesartan caused a modest decrease in plasma Ang II. Our findings in mRen2.Lewis hypertensive rats do not corroborate those studies or the Agata *et al.*[2] previous report in stroke-prone SHR. In the Agata *et al.* study, a 4-week administration of olmesartan reversed cardiac hypertrophy and lowered blood pressure in association with nonsignificant increases in circulating Ang II levels. Although the duration of the treatment period and the doses of olmesartan employed in our study and the Agata *et al.*[2] study were similar, differences in findings may be related to the use of stroke-prone SHR vs. congenic mRen2.Lewis hypertensive rats. Although Agata *et al.*[2] attributed the effects on circulating Ang II to an inhibitory effect of increased Ang-(1–7) on ACE activity, their hypothesis was not substantiated by our current study through direct measure of ACE activity. The majority of studies implicating Ang-(1–7) inhibitory effects on ACE activity were *in vitro* studies employing concentrations of Ang-(1–7) (μmol/L) greater than physiological levels[5,6] or the concentration of the heptapeptide reached in our studies after $AT_1$-R blockade (pmol/L). Furthermore, the relative increase of Ang II to Ang I as well as the relative

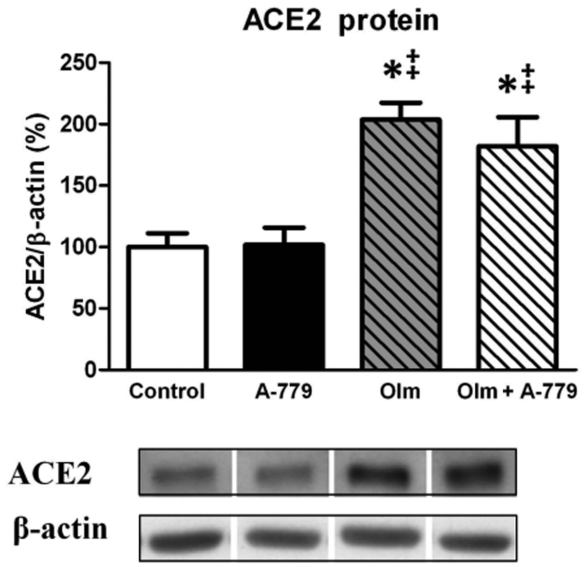


**Figure 4.** Effects of $AT_1$ and *mas* receptor antagonists on renal cortical angiotensin converting enzyme 2 (ACE2) protein expression. Values are expressed as mean ± SEM. n=7 per group. Abbreviations: A-779, *mas* receptor antagonist [D-ALA$^7$]-Ang-(1–7); Olm, olmesartan; $^*P < 0.05$ vs. control; $^‡P < 0.05$ vs. A-779.

decrease in Ang-(1–7) to Ang I in olmesartan-treated rats argues against a significant role of Ang-(1–7) in inhibiting the conversion of Ang I to Ang II by ACE in the circulation. In addition, a major difference between the 2 studies relates to their observation that A-799 caused a small but significant increase in the blood pressure (measured by tail-cuff) of olmesartan-treated rats that was associated with a 312% increases in plasma Ang II.[2] The more precise use of blood pressure telemetry in our study demonstrated that blockade of *mas* receptors does not oppose the antihypertensive effects induced by olmesartan and that A-799 has no additional effect on plasma and tissue Ang II levels or serum ACE activities. Therefore, our data demonstrate that the major antihypertensive mode of action of Ang II blockade with olmesartan is mediated through the $AT_1$-R.

In addition, we showed that the antihypertensive effects of olmesartan treatment are associated with marked suppression of serum aldosterone levels despite the presence of elevated plasma Ang I and Ang II levels. Thus, olmesartan appears to confer sustained aldosterone suppression over several weeks of administration, which is consistent with the effect of valsartan in patients with heart failure[32] but not diabetes mellitus.[33]

Concomitant blockade of $AT_1$ and *mas* receptors resulted in an additional augmentation in PRC when compared with $AT_1$-R antagonism alone. This is a new finding implicating a counterbalancing role for Ang-(1–7) in the control of renin release. Furthermore, the effect of *mas* receptor blockade on renin release was not observed in rats not treated with olmesartan, suggesting that renin activation may be essential for initiation of *mas* receptor–mediated effects. Importantly, $AT_2$-Rs inhibit renal renin production in salt-depleted rats treated with valsartan.[34] The inhibitory effect of $AT_2$-R on renin production was mediated through nitric oxide (NO), and the combined inhibition of $AT_2$-R and NO synthase causes no additional increase in renal renin. Because the majority of Ang-(1–7) renal actions are mediated through the NO pathway, it is possible that renal *mas* receptor activation leads to NO production and renin inhibition under the condition of concomitant $AT_1$-R blockade. In support of this hypothesis, we point to our recent findings of reduced renal *mas* protein expression in SHR fed a high-salt diet that was associated with increased plasma renin concentration.[35] Moreover, β-1 receptor antagonism reduced renin release and corrected the reduced *mas* receptor expression due to a high-salt diet while increasing expression of neuronal NO synthase.

We further demonstrated that kidney Ang II decreased following $AT_1$-R antagonism in both the absence and presence of *mas* receptor blockade. Numerous reports demonstrated $AT_1$-R–mediated Ang II uptake as well as stimulation of angiotensinogen[36,37] in the kidneys, which explains, at least in part, the dissociation between the kidney and plasma Ang II levels after $AT_1$-R blockade. Interestingly, we observed no significant change in renal Ang-(1–7) levels despite a significant fall in renal Ang II in the olmesartan-treated rats. That prompted us to investigate the activity of enzymes involved in the Ang-(1–7) formation and metabolism. We first demonstrated a significant increase in ACE2 protein in kidney cortex membranes of both groups treated with olmesartan, confirming tonic downregulation of ACE2 mediated by $AT_1$-R in rats harboring the m*Ren2* gene. However, HPLC measurement of ACE2 activities revealed only a tendency for an increase in ACE2-mediated conversion of Ang II to Ang-(1–7) in olmesartan-treated groups when compared with either vehicle- or A-779–treated rats. The uncoupling between the ACE2 gene, protein, and activity has been reported earlier.[3,38,39] However, previous studies from our laboratory[3,16] and others[40] showed that short-term blockade of $AT_1$-R induces an increase in ACE2 protein and activity. It is noteworthy to mention that Lew *et al.*[41] observed the presence of an endogenous inhibitor in plasma of healthy volunteers, upon which removal of the ACE2 activity was detected. In addition, the presence of

**Table 2.** Angiotensin converting enzyme (ACE), ACE2, and neprilysin (NEP) activities in kidney of mRen2.Lewis rats treated with $AT_1$ and *mas* receptor antagonists

| Enzyme | Control | A-779 | OLM | OLM + A-779 |
|---|---|---|---|---|
| ACE, nmol/mg/min | 1.9 ± 0.6 | 2.5 ± 0.3 | 2.4 ± 0.9 | 2.0 ± 0.8 |
| ACE2, fmol/mg/min | 32.0 ± 1.7 | 30.6 ± 1.0 | 36.7 ± 1.7 | 34.4 ± 1.9 |
| NEP, fmol/mg/min | 95.5 ± 2.9 | 105.0 ± 4.1 | 100.9 ± 4.7 | 97.5 ± 4.3 |

Values are mean ± SEM. A-779, *mas* receptor antagonist [D-ALA$^7$]-Ang-(1–7).
Abbreviation: OLM, olmesartan.

endogenous antibodies with inhibitory ACE2 activity has been shown in patients with vasculopathy and increased ACE2 enzyme protein.[38] Thus, it is tempting to speculate that increased ACE2 in response to longer exposure to $AT_1$-R antagonist may stimulate the production of ACE2 antibodies or inhibitors, which may eventually reduce its activity. In addition, previous *in vitro* reports showed that Ang-(1–7) prevented Ang II–induced decrease in ACE2 mRNA expression and activity. The effects were abolished by the Ang-(1–7) *mas* receptor antagonist A-799.[11] In this study on renal tissue, we did not observe any effects of *mas* receptor blockade with respect to ACE2 protein and activity in either vehicle- or olmesartan-treated rats. Further studies are thus required to determine whether absence of ACE2 change in kidney in response to A-799 administration implies a differential involvement of *mas* receptors in ACE2 regulation in diverse organs.

We also investigated the activity of ACE and NEP because ACE metabolizes Ang-(1–7) whereas NEP forms it from Ang I and metabolizes it to smaller fragments, mainly Ang-(1–4) in the kidney.[18] Thus, inhibition of ACE or NEP could lead to Ang-(1–7) accumulation in the kidney of olmesartan-treated animals. However, there was no change in these enzyme activities in the kidney cortex membranes after olmesartan treatment, and therefore other enzymes relevant for Ang-(1–7) formation, which were not examined in this study, may contribute to the preserved renal Ang-(1–7). More specifically, prolyl endopeptidase, whose activity was confirmed in different region of nephron, uses both Ang I and Ang II to form Ang-(1–7).[42–44] In this connection, a detailed study by Velez *et al.*[19] on angiotensin metabolism in cultured human glomerular podocytes and mesangial cells showed that prolyl endopeptidase rather than NEP contributes to Ang-(1–7) formation.

The absence of any changes in plasma or tissue Ang-(1–7) content in our experiments using a specific *mas* receptor antagonist in both vehicle and olmesartan treatment suggests that the *mas* receptor does not mediate tissue Ang-(1–7) uptake. Thus, it is less likely that renal *mas* receptor–mediated Ang-(1–7) uptake is responsible for the preserved renal Ang-(1–7) levels in kidneys in which less Ang II is available for its conversion through ACE2. In keeping with this interpretation, a recent study suggests that megalin, the receptor expressed abundantly in the brush border of the proximal tubules, mediates the nonspecific uptake of different peptides, including Ang-(1–7).[45] Moreover, $AT_1$-R regulates megalin expression in cultured proximal tubular cells,[46] whereas 3 weeks of $AT_1$-R blockade upregulated reduced megalin expression in hypertensive kidney of transgenic m[Ren2]27 hypertensive rats.[47] Although further studies will validate the pathophysiological relevance of megalin in renal uptake of Ang-(1–7) in response to $AT_1$-R blockade, the higher renal Ang-(1–7)/Ang II ratio found in our study in response to RAS blockade supports the concept that renal Ang-(1–7) contributes to the well-documented beneficial renal effects of ARBs.

In summary, this study shows that the potent antihypertensive and antihypertrophic effects of olmesartan are comparable with those obtained with other $AT_1$-R antagonists that are not capable of effectively suppressing serum aldosterone in the face of increased circulating Ang II. Suppression of renal Ang II content without changes in renal Ang-(1–7) after $AT_1$-R blockade may be a mechanism associated with the known renoprotective effect of $AT_1$-R blockade. The primary effects of olmesartan are not altered by concomitant administration of an Ang-(1–7) receptor antagonist, a finding that underscores a primary role of the $AT_1$-R rather than the *mas* receptor in the modulation of blood pressure. In addition, we show a mild but significant effect of Ang-(1–7) in the modulation of renal renin secretion that may be mediated by a non-$AT_1$-R.

## SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal of Hypertension* (http://ajh.oxfordjournals.org).

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## DISCLOSURE

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