*Original Article*

# Olmesartan Is an Angiotensin II Receptor Blocker with an Inhibitory Effect on Angiotensin-Converting Enzyme

Jun AGATA[1)], Nobuyuki URA[1)], Hideaki YOSHIDA[1)], Yasuyuki SHINSHI[1)], Haruki SASAKI[1)], Masaya HYAKKOKU[1)], Shinya TANIGUCHI[1)], and Kazuaki SHIMAMOTO[1)]

**Angiotensin II receptor blockers (ARBs) are widely used for the treatment of hypertension. It is believed that treatment with an ARB increases the level of plasma angiotensin II (Ang II) because of a lack of negative feedback on renin activity. However, Ichikawa (*Hypertens Res* 2001; 24: 641–646) reported that long-term treatment of hypertensive patients with olmesartan resulted in a reduction in plasma Ang II level, though the mechanism was not determined. It has been reported that angiotensin 1-7 (Ang-(1-7)) potentiates the effect of bradykinin and acts as an angiotensin-converting enzyme (ACE) inhibitor. It is known that ACE2, which was discovered as a novel ACE-related carboxypeptidase in 2000, hydrolyzes Ang I to Ang-(1-9) and also Ang II to Ang-(1-7). It has recently been reported that olmesartan increases plasma Ang-(1-7) through an increase in ACE2 expression in rats with myocardial infarction. We hypothesized that over-expression of ACE2 may be related to a reduction in Ang II level and the cardioprotective effect of olmesartan. Administration of 0.5 mg/kg/day of olmesartan for 4 weeks to 12-week-old stroke-prone spontaneously hypertensive rats (SHRSP) significantly reduced blood pressure and left ventricular weight compared to those in SHRSP given a vehicle. Co-administration of olmesartan and (D-Ala$^7$)–Ang-(1-7), a selective Ang-(1-7) antagonist, partially inhibited the effect of olmesartan on blood pressure and left ventricular weight. Interestingly, co-administration of (D-Ala$^7$)–Ang-(1-7) with olmesartan significantly increased the plasma Ang II level (453.2±113.8 pg/ml) compared to olmesartan alone (144.9±27.0 pg/ml, $p<0.05$). Moreover, olmesartan significantly increased the cardiac ACE2 expression level compared to that in Wistar Kyoto rats and SHRSP treated with a vehicle. Olmesartan significantly improved cardiovascular remodeling and cardiac nitrite/nitrate content, but co-administration of olmesartan and (D-Ala$^7$)–Ang-(1-7) partially reversed this anti-remodeling effect and the increase in nitrite/nitrate. These findings suggest that olmesartan may exhibit an ACE inhibitory action in addition to an Ang II receptor blocking action, prevent an increase in Ang II level, and protect cardiovascular remodeling through an increase in cardiac nitric oxide production and endogenous Ang-(1-7) *via* over-expression of ACE2. (*Hypertens Res* 2006; 29: 865–874)**

***Key Words*: olmesartan, angiotensin-(1-7), angiotensin II, angiotensin-converting enzyme 2**

## Introduction

Angiotensin-converting enzyme (ACE) catalyzes the formation of angiotensin II (Ang II) and thereby plays a key role in cardiorenal regulation and blood pressure (BP) control. Overactivity of the renin-angiotensin system contributes to the pathophysiology of hypertension and heart failure, which is

From the [1)]Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan.
Address for Reprints: Jun Agata, M.D., Ph.D., Second Department of Internal Medicine, Sapporo Medical University School of Medicine, S-1, W-16, Chuo-ku, Sapporo 060–8543, Japan. E-mail: agatajun@yahoo.co.jp
Received April 10, 2006; Accepted in revised form July 19, 2006.

reflected in the use of ACE inhibitors in the treatment of these conditions. In 2000, a novel ACE-related carboxypeptidase, ACE2, was discovered and was characterized as an enzyme similar to ACE (*1*). ACE2 is a membrane-associated carboxypeptidase that is highly expressed in the heart, kidney and testis (*1*). It is known that ACE2 hydrolyzes angiotensin I (Ang I) to angiotensin 1-9 (Ang-(1-9)) and also Ang II to angiotensin 1-7 (Ang-(1-7)) (*2*). In turn, Ang-(1-9) can be converted to Ang-(1-7) by the action of two endopeptidases, neutral endopeptidase and prolyl endopeptidase. Therefore, ACE2 facilitates the production of Ang-(1-7) by two separate pathways (*3*) that depart from the classical system. A potential role for ACE2 in the cardioprotective effect of Ang-(1-7) was demonstrated by Loot *et al* (*4*). Ang-(1-7) exerts direct vasodilatory effects *via* non–angiotensin II type 1 ($AT_1$) and non–angiotensin II type 2 ($AT_2$) receptors, possibly by stimulating bradykinin and NO release (*3*, *5*). Moreover, Ang-(1-7) antagonizes the pressor effects of Ang II, suggesting that it may cause vasodilation indirectly by acting as an $AT_1$ receptor antagonist (*6*, *7*). In addition, Ang-(1-7) potentiates bradykinin, either *via* an $AT_2$ receptor–dependent mechanism or through inhibition of ACE (*6*, *8*, *9*). Recent studies have also shown that Ang-(1-7), like ACE inhibitors, potentiates bradykinin by inhibiting desensitization of its receptor (*10–12*).

Olmesartan is a novel $AT_1$ receptor blocker (ARB) synthesized by Sankyo Co., Ltd. (Tokyo, Japan). The antihypertensive effect of olmesartan in spontaneously hypertensive rat (SHR) is about 30 times greater than that of losartan potassium, another drug of the same class, and nearly equal to that of candesartan cilexetil. Ichikawa reported that long-term treatment of hypertensive patients with olmesartan resulted in a reduction of the plasma Ang II level (*13*), despite the fact that several types of ARBs have been shown to increase both plasma renin activity and plasma Ang II concentrations in hypertensive patients (*14–16*). However, the mechanism underlying the failure of olmesartan to increase the plasma Ang II levels remains uncertain. Recently, Ishiyama reported that olmesartan increased ACE2 expression in the remodeling heart after myocardial infarction, which theoretically could contribute to the beneficial effects of ARB by facilitating increased cardiac Ang-(1-7) formation (*17*). It has also been reported that Ang-(1-7) inhibits the ACE C-domain and potentiates bradykinin by acting as an ACE inhibitor (*18*). We therefore hypothesized that olmesartan, in addition to blocking $AT_1$ receptors, inhibits ACE by increasing the expression of ACE2 and thereby enhancing the production of Ang-(1-7), since Ang-(1-7) can inhibit ACE activity, as mentioned above. In order to evaluate this hypothesis, we investigated the effects of olmesartan on ACE2 expression and Ang-(1-7).

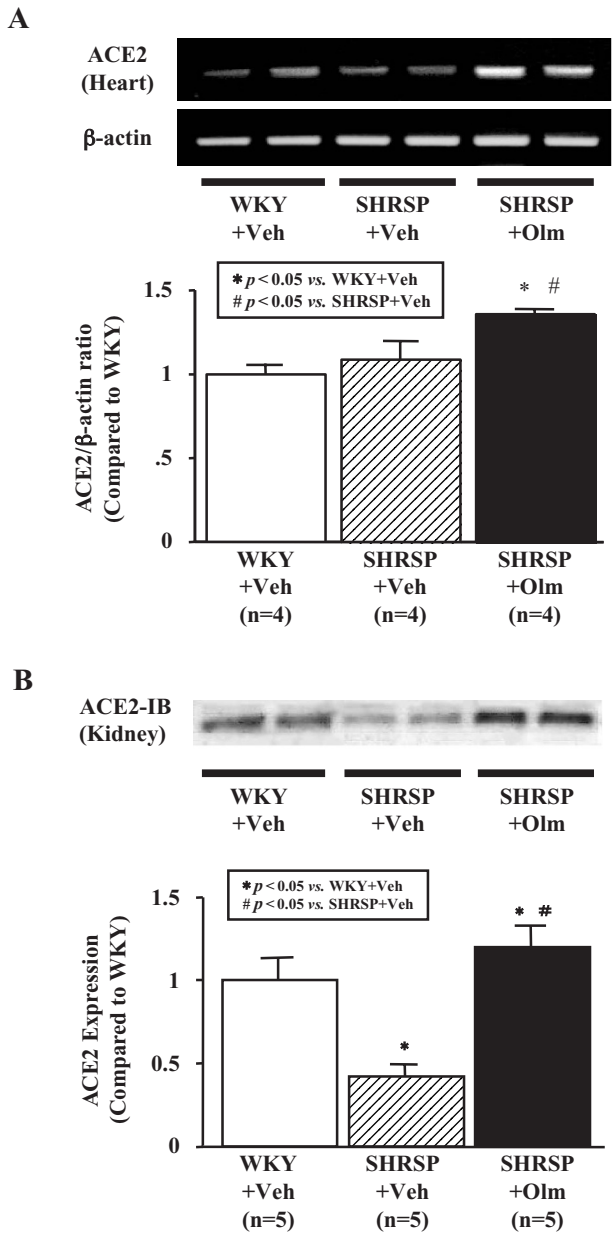


**Fig. 1.** *Effect of olmesartan treatment for 4 weeks in SHRSP on cardiac ACE2 mRNA expression (A) and renal ACE2 protein level (B).* $^{*}p < 0.05$ vs. *WKY treated with vehicle (WKY+Veh);* $^{\#}p < 0.05$ vs. *SHRSP treated with vehicle (SHRSP+Veh). SHRSP+Olm, SHRSP treated with olmesartan.*

## Methods

### Animals

Male Wistar-Kyoto rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP) were purchased from

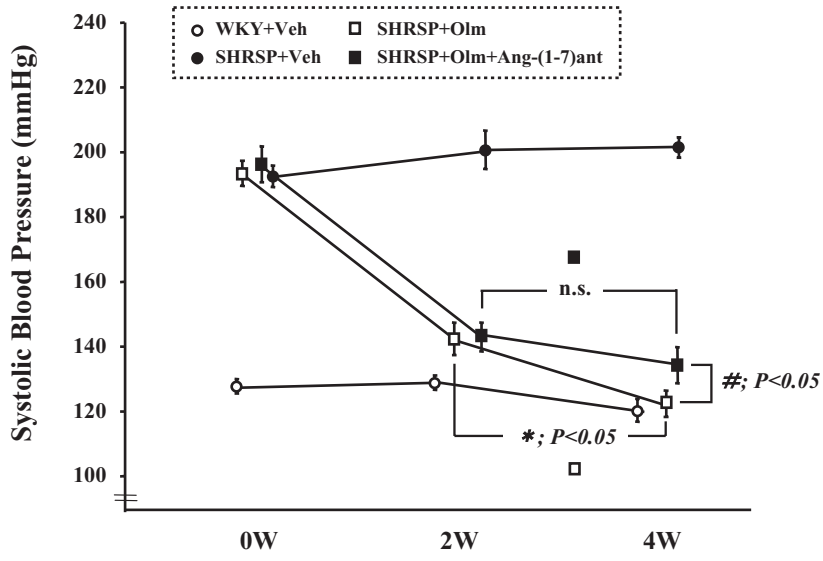


**Fig. 2.** *Effect of olmesartan (0.5 mg/kg/day) and/or Ang-(1-7) antagonist (0.5 mg/kg/day) on systolic blood pressure in SHRSP and WKY (*n*=13–17). Values are the means±SEM.* *p*<0.05 for the effects of olmesartan in SHRSP at 2 weeks and 4 weeks.* #p*<0.05 for the effects of the Ang-(1-7) antagonist during the treatment with olmesartan. WKY+Veh, WKY treated with vehicle; SHRSP+Veh, SHPRSP treated with vehicle; SHRSP+Olm, SHRSP treated with olmesartan; SHRSP+Olm+Ang-(1-7)ant, SHRSP treated with olmesartan and the Ang-(1-7) antagonist.*

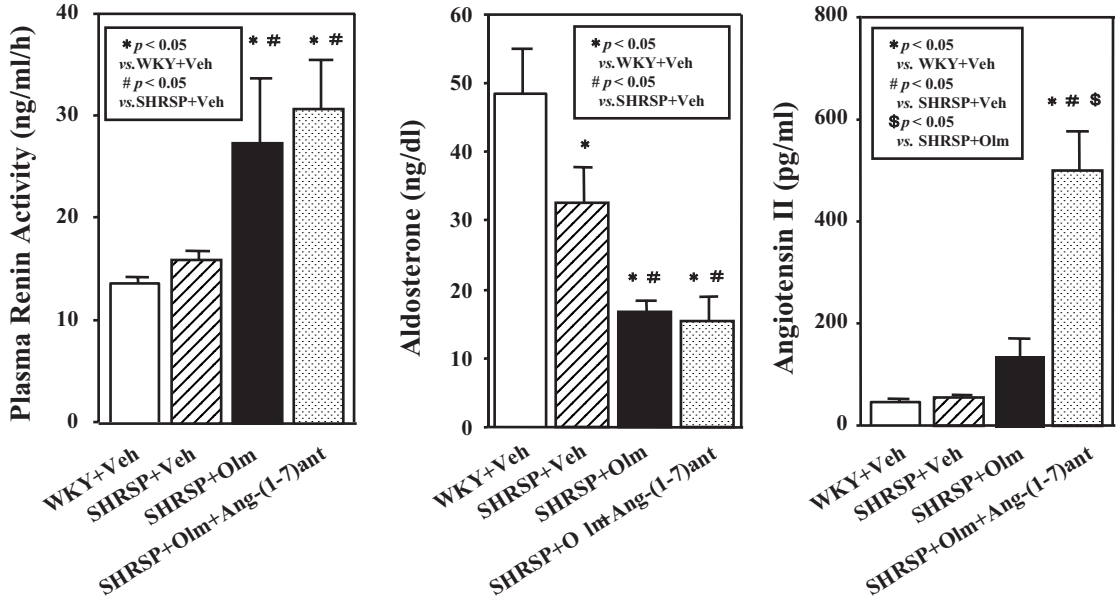


**Fig. 3.** *Effects of olmesartan and the Ang-(1-7) antagonist on circulating levels of aldosterone and angiotensin II. Values are the means±SEM.* *p*<0.05* vs. *WKY treated with vehicle (WKY+Veh);* #p*<0.05* vs. *SHRSP treated with vehicle (SHRSP+Veh);* $p*<0.05* vs. *SHRSP treated with olmesartan (SHRSP+Olm). SHRSP+Olm+Ang-(1-7)ant, SHRSP treated with olmesartan and the Ang-(1-7) antagonist.*

Hoshino Examination Animal Breeding Corporation (Saitama, Japan). The rats were housed in animal quarters with a 12-h light/dark cycle and were provided a standard rat chow containing 60% vegetable starch, 5% fat, and 24% protein (Oriental Yeast Co., Tokyo, Japan) and distilled water *ad libitum*. All experiments were conducted in accordance with

**Table 1. Blood Pressure, Pulse Rate and Cardiac Hypertrophic Parameters among Groups**

| | WKY+Veh ($n$=13) | SHRSP+Veh ($n$=17) | SHRSP+Olm ($n$=15) | SHRSP+Olm+Ang-(1-7)ant ($n$=13) |
|---|---|---|---|---|
| BW (g) | 317.5±3.5 | 283.8±2.8* | 272.9±3.8*,# | 278.3±2.6* |
| SBP (mmHg) | 120.1±3.5 | 202.5±3.7* | 125.8±3.1# | 137.0±4.1#,$ |
| PR (/min) | 297.6±10.5 | 351.1±10.0* | 393.6±7.9*,# | 396.5±9.6*,# |
| HW (g) | 1.09±0.03 | 1.18±0.02* | 0.91±0.02*,# | 0.96±0.01*,# |
| HW/BW (g/kg) | 3.42±0.07 | 4.16±0.05* | 3.33±0.06# | 3.45±0.04# |
| LVW (g) | 0.78±0.02 | 0.86±0.01* | 0.64±0.01# | 0.69±0.01#,$ |
| LVW/BW (g/kg) | 2.45±0.05 | 3.04±0.03* | 2.34±0.04# | 2.48±0.03#,$ |

Values are mean±SEM. *$p<0.05$ *vs*. WKY+Veh, #$p<0.05$ *vs*. SHRSP+Veh, $$p<0.05$ *vs*. SHRSP+Olm. BW, body weight; SBP, systolic blood pressure; PR, pulse rate; HW, heart weight; LVW, left ventricle weight. WKY+Veh, WKY treated with vehicle; SHRSP+Veh, SHPRSP treated with vehicle; SHRSP+Olm, SHRSP treated with olmesartan; SHRSP+Olm+Ang-(1-7)ant, SHRSP treated with olmesartan and the Ang-(1-7) antagonist.

the NIH Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy Press, Washington, DC, USA). Permission for the study was granted by the Ethics Committee on Animal Experiments, Sapporo Medical University.

## Effects of Chronic Infusion of Olmesartan and Ang-(1-7) Antagonist in SHRSP

To investigate the potential roles of Ang-(1-7) during treatment with olmesartan in SHRSP, rats of 12 weeks of age were divided into four experimental groups ($n$=13–17 in each group): 1) vehicle treatment for WKY (WKY+Veh group), 2) vehicle treatment for SHRSP (SHRSP+Veh group), 3) olmesartan (0.5 mg/kg/day, Sankyo Co., Ltd., Tokyo, Japan) treatment for SHRSP (SHRSP + Olm group), and 4) olmesartan (0.5 mg/kg/day, Sankyo Co., Ltd.) with (D-Ala$^7$)–Ang-(1-7) (0.5 mg/kg/day, Bachem California Inc., Torrance, USA) treatment for SHRSP (SHRSP + Olm + Ang-(1-7)ant group). Olmesartan and/or (D-Ala$^7$)–Ang-(1-7) was infused subcutaneously for 4 weeks by osmotic mini pumps (ALZET #2004; Alza Corp., Palo Alto, USA). Sodium hydrogencarbonate (2.5% solution) was used as a vehicle. The dose of Ang-(1-7) antagonist was chosen on the basis of a previous study (*19*, *20*). BP and pulse rate were measured by the tail-cuff method every 2 weeks, and rats were sacrificed at the end of 4 weeks. Blood samples were collected for measurements of aldosterone and Ang II. Plasma renin activity (PRA), plasma aldosterone concentration (PAC) and Ang II level were measured by radioimmunoassay or the two-antibody method (MBC, Inc., Tokyo, Japan). The heart of each rat was extracted, and heart weight (HW) and left ventricular weight (LVW) were measured, and then the HW/body weight (BW) and LVW/BW values were calculated. After measurement of HW and LVW, the extracts were frozen in liquid nitrogen and stored at −80°C until measurements of nitrite/nitrate (NOx) by a colorimetric assay and of ACE2 and β-actin expression by the reverse transcriptase–polymerase chain reaction (RT-PCR) method described previously (*17*). The primers used are described below. Other samples of the excised hearts were fixed with formalin, and paraffin-embedded 4-μm–thick slices were stained with Sirius red for histological analysis. NOx measurements in the heart tissue extracts were made using a colorimetric assay with a griess reagent kit (Dojindo Lab., Tokyo, Japan).

## Expressions of ACE2 in the Heart and Kidney

One μg of RQ1 DNAase-treated total RNA isolated from the left ventricle with the Trizol reagent (GIBCO BRL, Gaithersburg, USA) was quantified by ultraviolet spectroscopy, and a RT-PCR assay was performed using primers for ACE2, 5′-GTGCACAAAGGTGACAATGG-3′ and 5′-ATGCGGGGT CACAGTATGTT-3′. Amplification conditions for measurement of ACE2 mRNA were as follows: 30 cycles of denaturation at 94°C for 60 s, annealing at 60°C for 60 s, and elongation at 72°C for 60 s; followed by a final elongation step at 72°C for 7 min. Amplification products were separated on a 6% polyacrylamide gel, visualized using a PhosphorImager, and quantified by computerized densitometry. The kidney extract was prepared for Western blot analysis using a specific antibody for ACE2 (Santa Cruz Biotechnology, Inc., Santa Cruz, USA). All values were normalized by arbitrarily setting the integrated densitometric values of WKY rats to 1.0.

## Histological Examination and Evaluation of Cardiovascular Remodeling

Histological examination was done as described previously (*21*, *22*). To evaluate the coronary arterial wall thickness and perivascular fractional fibrosis, we scanned short-axis images of intramyocardial arteries at ×200 magnification. To assess any thickening of the arterial wall and perivascular fibrosis, trans-sectional images of the area of the total small arteriolar lumen ≤ $2 \times 10^4$ μm$^2$ were studied. The wall-to-lumen ratio (area of the vessel wall divided by area of the total blood ves-

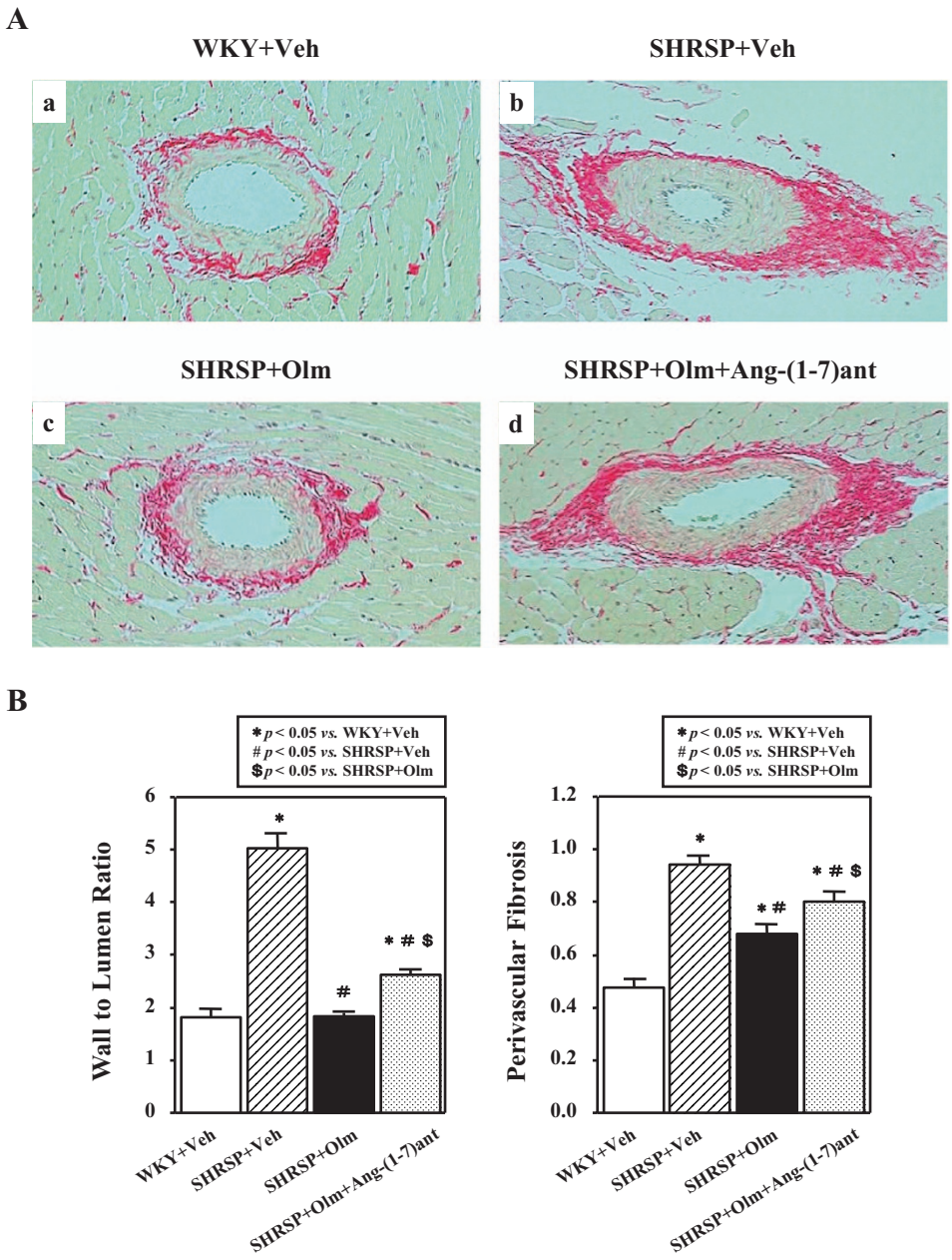


**Fig. 4.** *A: Micrographs of coronary arterioles with Sirius red stain for WKY treated with vehicle (WKY+Veh) (a), SHRSP treated with vehicle (SHRSP+Veh) (b), SHRSP treated with olmesartan (SHRSP+Olm) (c), and SHRSP treated with olmesartan and the Ang-(1-7) antagonist (SHRSP+Olm+Ang-(1-7)ant) (d). B: Effects of chronic olmesartan and/or Ang-(1-7) antagonist treatment on cardiovascular remodeling in SHRSP: wall-to-lumen ratio (left) and perivascular fibrosis (right).* $^{*}p < 0.05$ vs. *WKY+Veh;* $^{\#}p < 0.05$ vs. *SHRSP+Veh;* $^{\$}p < 0.05$ vs. *SHRSP+Olm.*

sel lumen) was determined. The area of fibrosis immediately surrounding the blood vessels was calculated, and perivascular fibrosis was determined as the ratio of the area of fibrosis surrounding the vessel wall to the total area of the vessel. In order to evaluate these parameters, we chose 8 different intramyocardial arteries in each heart, and analyzed them using Scion Image software (ver. 4.3.0.2).

## Statistical Analysis

All data are expressed as the means±SEM. Statistical analysis was performed by ANOVA followed by Fisher's test for multiple comparisons. Values of $p<0.05$ were considered to indicate statistical significance.

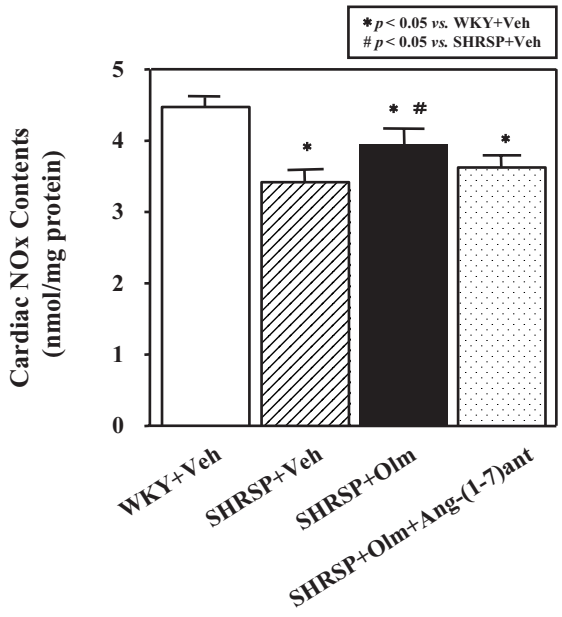


**Fig. 5.** *Effects of chronic olmesartan and/or Ang-(1-7) antagonist treatment on cardiac NOx content in SHRSP.* *p < *0.05* vs. *WKY treated with vehicle (WKY+Veh);* #p < *0.05* vs. *SHRSP treated with vehicle (SHRSP+Veh). SHRSP+Olm, SHRSP treated with olmesartan; SHRSP+Olm+Ang-(1-7)ant, SHRSP treated with olmesartan and the Ang-(1-7) antagonist.*

## Results

### Expression of ACE2

There was no significant difference in the cardiac ACE2 mRNA expression between WKY and SHRSP. Cardiac ACE2 mRNA levels were 1.4- and 1.3- fold ($p<0.05$, respectively) larger in SHRSP treated with olmesartan than in WKY treated with a vehicle or SHRSP treated with a vehicle (Fig. 1A). The renal ACE2 protein level in SHRSP was significantly lower than that in WKY ($p<0.05$), and olmesartan significantly increased the renal ACE2 protein level ($p<0.05$) (Fig. 1B).

### Blood Pressure

BPs in the SHRSP+Veh, SHRSP+Olm, and SHRSP+Olm+Ang-(1-7)ant groups (192±3 mmHg, 193±4 mmHg and 199±5 mmHg, respectively) were significantly higher than that in the WKY+Veh group at 12 weeks of age (127±2 mmHg, $p<0.01$). BPs in the SHRSP+Olm and SHRSP+Olm+Ang-(1-7)ant group (141±3 mmHg and 145±3 mmHg, respectively) were significantly lower than that in the SHRSP+Veh group (199±4 mmHg, $p<0.05$), but there was no significant difference between BPs in the SHRSP+Olm and SHRSP+Olm+Ang-(1-7)ant groups until 2 weeks after the start of treatment. At the end of this study, BPs in the SHRSP+Olm and SHRSP+Olm+Ang-(1-7)ant groups (125±3 mmHg and 137±4 mmHg, respectively) were significantly lower than that in the SHRSP+Veh group (202±4 mmHg, $p<0.05$), and the BP in the SHRSP+Olm group was significantly lower ($p<0.05$) than that in the SHRSP+Olm+Ang-(1-7)ant group. Moreover, olmesartan treatment resulted in a significant reduction in BP at 4 weeks compared to that at 2 weeks, although there was no significant difference between BPs in the SHRSP+Olm+Ang-(1-7)ant group at 2 and 4 weeks (Fig. 2).

### PRA, PAC, and Ang II Level

PRA in the SHRSP+Olm and SHRSP+Olm+Ang-(1-7)ant groups (27.3±6.4 ng/ml/h and 30.6±4.8 ng/ml/h, respectively) were significantly higher than those in the WKY+Veh and SHR+Veh groups (13.5±0.7 ng/ml/h and 15.8±1.0 ng/ml/h, respectively, $p<0.05$), but there was no significant difference between the levels in the SHRSP+Olm and SHRSP+Olm+Ang-(1-7)ant groups. PAC in the SHRSP+Olm and SHRSP+Olm+Ang-(1-7)ant groups (17.3±1.9 ng/dl and 16.4±3.7 ng/dl, respectively) were significantly lower than those in the WKY+Veh and SHR+Veh groups (48.5±6.4 ng/dl and 32.2±4.2 ng/dl, respectively, $p<0.05$), but there was no significant difference between the levels in the SHRSP+Olm and SHRSP+Olm+Ang-(1-7)ant groups. There were no significant differences between plasma Ang II levels in the WKY+Veh, SHRSP+Veh and SHRSP+Olm groups (44.8±7.9 pg/ml, 46.8±11.4 pg/ml and 144.9±27.0 pg/ml, respectively), but co-administration of olmesartan and an Ang-(1-7) antagonist significantly increased the plasma Ang II level (453.2±113.8 pg/ml) compared to those in the other groups ($p<0.05$) (Fig. 3).

### Cardiovascular Remodeling and Cardiac NOx Content

BW in SHRSP was significantly lower than that in WKY. HW, LVW, HW/BW and LVW/BW in SHRSP were significantly higher than those in WKY. Four weeks of treatment with olmesartan reduced these parameters of cardiac hypertrophy. Co-administration of olmesartan and an Ang-(1-7) antagonist partially blocked the anti–cardiac hypertrophic effect, especially in LVW (Table 1). The wall-to-lumen ratio and perivascular fibrosis were significantly increased in the SHRSP+Veh group (5.0±0.3 and 0.94±0.04, respectively) compared with those in the WKY+Veh group (1.8±0.2 and 0.48±0.04, respectively, $p<0.05$). Olmesartan significantly reduced these parameters (1.8±0.1 and 0.68±0.03, respectively, $p<0.05$ *vs*. SHRSP+Veh), but co-administration of olmesartan and (D-Ala$^7$)–Ang-(1-7) partially reversed these parameters for cardiovascular remodeling (2.6±0.1 and 0.80±0.04, respectively, $p<0.05$ *vs*. SHRSP+Olm) (Fig. 4).

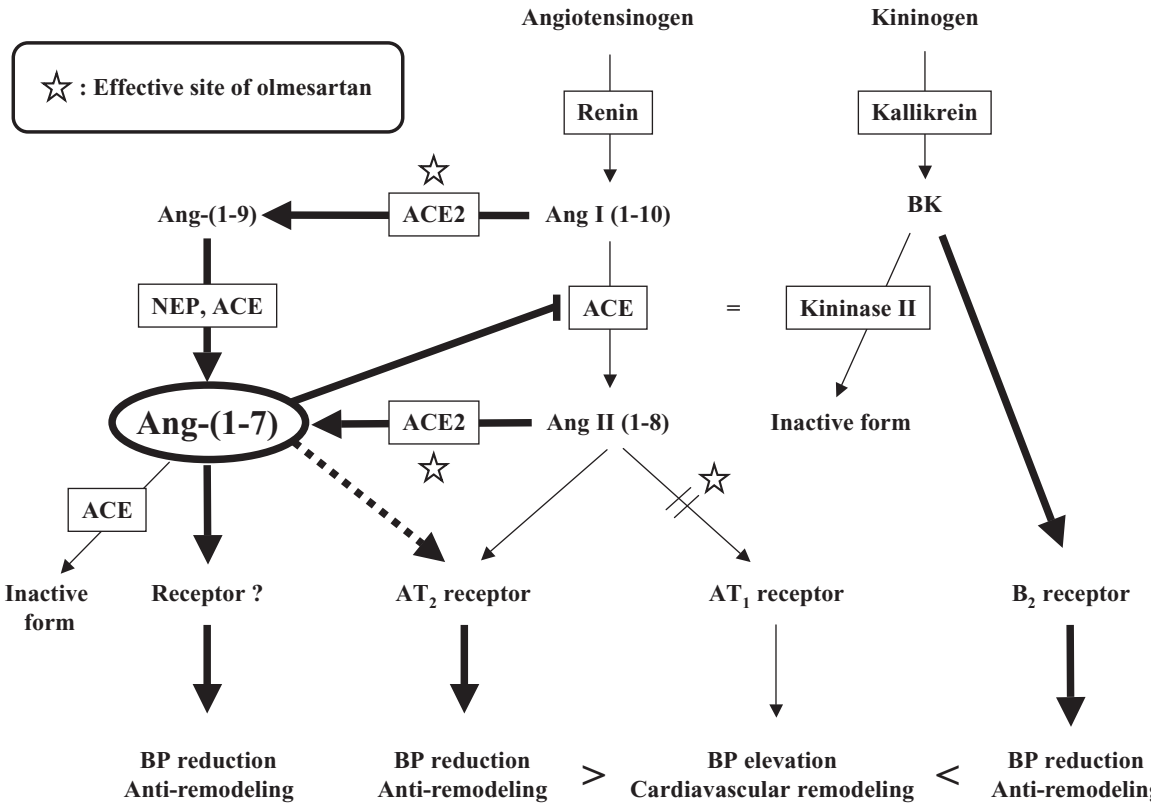


**Fig. 6.** *Scheme of the linkage of the renin-angiotensin system and kallikrein-kinin system. Angiotenisn-converting enzyme 2 (ACE2) converts Ang I to Ang-(1-9) and Ang II to Ang-(1-7). Ang-(1-9) is finally converted to Ang-(1-7) by ACE or neutral endopeptidase (NEP). Ang-(1-7) has several effects, such as an ACE inhibitory effect, a vasodilating effect and an $AT_2$ receptor stimulating effect. Olmesartan increases the ACE2 expression level and may increase the level of Ang-(1-7). The inhibition of ACE by Ang-(1-7) inhibits kininase II and enhances the effect of bradykinin (BK). Olmesartan may have dual actions as an angiotensin II receptor blocker and an ACE inhibitor through an increase in Ang-(1-7)* via *over-expression of ACE2.*

Cardiac NOx content in SHRSP (3.4±0.2 mmol/mg protein) was significantly lower than that in WKY (4.5±0.1 mmol/mg protein, $p<0.05$), and 4 weeks of treatment with olmesartan significantly increased cardiac NOx content (3.9±0.2 mmol/mg protein, $p<0.05$), but co-administration of olmesartan and an Ang-(1-7) antagonist partially, but significantly blocked this olmesartan effect (3.6±0.2 mmol/mg protein, $p<0.05$) (Fig. 5).

## Discussion

This is the first report that olmesartan has an endogenous ACE inhibitory effect through an increase in Ang-(1-7) *via* over-expression of ACE2. This may be one of the mechanisms by which olmesartan achieves its strong BP reduction and anti–cardiovascular remodeling effects.

### Over-Expression of ACE2 by Olmesartan

ACE2 is a carboxypeptidase that is insensitive to known ACE inhibitors (*2*, *23–25*) and exhibits a high catalytic efficiency for the generation of Ang-(1-7) from Ang II (*26*). Ablation of ACE2 in mice causes severe cardiac dysfunction, a finding that suggests an important function of ACE2 as a regulator of cardiac function (*25*). Ishiyama *et al*. reported that ACE2 is unchanged during the process of ventricular remodeling in post-myocardial infarction but that olmesartan increases ACE2 mRNA (*17*). In agreement with their results, we found a significant increase in the ACE2 mRNA expression level in SHRSP treated with olmesartan (Fig. 1). Since PD123319, an $AT_2$ receptor antagonist, had no effect on the olmesartan-induced over-expression of ACE2 in the study by Ishiyama *et al*., Ang II itself may regulate ACE2 expression but not $AT_2$ receptors (*17*). This speculation is supported by the results of a study in which Ang II down-regulated ACE2 mRNA in cerebellar astrocytes in culture (*27*). However, the mechanism by which olmesartan increases the expression level of ACE2 is still unclear, and further studies will be needed to clarify this mechanism.

## ACE Inhibitory Effect of Olmesartan

The BP reduction and organ protection achieved by ARBs seem to be due to blocking of the $AT_1$ receptors. It is believed that administration of ARBs increases the Ang II level because of a lack of negative feedback on renin. Several studies have shown that most ARBs increase renin activity and/or plasma Ang II level (*28*, *29*). Increased Ang II binds to the $AT_2$ receptors, which is mostly opposite physiological effects against the $AT_1$ receptor. However, blocking of the $AT_1$ receptors by an ARB may be attenuated by increased Ang II, because some ARBs may be surmountable by Ang II. Interestingly, Ichikawa and Takayama reported that long-term treatment of hypertensive patients with olmesartan resulted in a reduction in the plasma Ang II level, but the mechanism of this effect was not clarified (*13*). In this study, olmesartan decreased PAC and increased PRA in the same manner as other ARBs, but the plasma Ang II level was slightly but not significantly increased by olmesartan (Fig. 3). Interestingly, co-administration of olmesartan and an Ang-(1-7) antagonist significantly increased the plasma Ang II level compared to olmesartan alone. Olmesartan also significantly increased the mRNA expression level of ACE2. It has been reported that Ang-(1-7), like quinaprilat and captopril, potentiates bradykinin by acting as an ACE inhibitor through inhibition of the ACE C-domain but not the N-domain (*18*). Moreover, Mendes *et al*. reported that chronic infusion of angiotensin-(1-7) reduced heart Ang II levels and increased cardiac ACE2 expression levels in rats (*30*). These findings indicate that olmesartan may inhibit ACE activity through an increase in endogenous Ang-(1-7) *via* increased expression of ACE2 in the manner of an ACE inhibitor (Fig. 6).

## Antihypertensive Effect of Olmesartan

In this study, an Ang-(1-7) antagonist partially blocked the BP-lowering effect of olmesartan, indicating that increased Ang-(1-7) through over-expression of ACE2 may be one of the mechanisms of the BP-lowering effect of olmesartan. Ang-(1-7) exerts direct vasodilatory effects by stimulating bradykinin and NO release (*3*, *5*) through inhibition of ACE (*6*, *8*, *9*), as mentioned above. It has been reported that ACE inhibitors, including Ang-(1-7), potentiate bradykinin not only through blockade of bradykinin hydrolysis but also by inhibiting desensitization of its receptor (*10–12*). Moreover, it has recently been reported that co-administration of candesartan and Ang-(1-7) caused a marked and sustained reduction in BP and that PD123319, which is an $AT_2$ receptor antagonist, attenuated the enhanced depressor response evoked by the Ang-(1-7)/candesartan combination in SHR (*31*). These results indicate that Ang-(1-7) evoked a depressor response during $AT_1$ receptor blockade *via* stimulation of the $AT_2$ receptor and a reduction in the breakdown of bradykinin.

Cardiovascular remodeling parameters such as LVW and coronary wall-to-lumen ratio were significantly reduced by olmesartan, but an Ang-(1-7) antagonist partially blocked these effects (Table 1 and Fig. 4). It was expected that one of the mechanisms for this blocking of the effects of olmesartan would be related to the inhibitory effect of Ang-(1-7) antagonists on BP reduction. However, increased Ang-(1-7) itself may also directly prevent cardiovascular remodeling through the potentiation of bradykinin, either *via* an $AT_2$ receptor–dependent mechanism or through the inhibition of ACE, because it is well known that bradykinin has an inhibitory effect on cardiac hypertrophy and fibrosis (*32*) (Fig. 6).

## Advantages of Olmesartan for the Treatment of Cardiovascular Disease

In this study, co-administration of an Ang-(1-7) antagonist with olmesartan partially blocked the effects of BP reduction and prevention of cardiac hypertrophy and vascular remodeling. Recently, Santos *et al*. reported that Ang-(1-7) is an endogenous ligand of the G-protein–coupled receptor Mas (*33*). This Mas receptor appears to also be involved in the anti-proliferative effect of Ang-(1-7) in vascular smooth muscle cells (*34*). It has been reported that Ang-(1-7) inhibits ACE activity (*18*) and may stimulate $AT_2$ receptors (*31*). These are unique mechanisms of olmesartan. The ACE-inhibitory effect of olmesartan can block the $AT_1$ receptors more efficiently because Ang II does not increase, and can also enhance the effect of bradykinin through the inhibition of kininase II. It is well known that increased bradykinin stimulates the NO-cGMP or prostaglandin-cAMP pathway. Both pathways may be important for reducing cardiac remodeling, such as hypertrophy or fibrosis (*32*). According to our results, olmesartan significantly increased cardiac NOx content, and an Ang-(1-7) antagonist inhibited this increase (Fig. 5). Moreover, the increased Ang-(1-7) itself may be related to the vasodilatory effect and organ-protective effects, such as the inhibition of vascular remodeling and cardiac hypertrophy (*35–37*) (Fig. 6).

## Study Limitations

Although we speculated that olmesartan may increase Ang-(1-7) through the increase in ACE2 expression, we did not measure Ang-(1-7) itself. However, the effects of olmesartan were partially blocked by the Ang-(1-7) antagonist. Moreover, it is unclear whether these effects are unique to olmesartan, because we did not study the effects of other ARBs. Further studies will be needed to clarify these points.

## Summary

Olmesartan is an ARB with an endogenous ACE inhibitory effect that is exerted through an increase in Ang-(1-7) *via* over-expression of ACE2. This new concept is very important to investigate the pleiotropic mechanisms of olmesartan in cardiovascular diseases.

## References


1. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ: A human homolog of angiotensin converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* 2000; **275**: 33238–33243.
2. Donoghue M, Hsieh F, Baronas E, *et al*: A novel angiotensin-converting enzyme–related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res* 2000; **87**: e1–e9.
3. Ferrario CM, Chappell MC, Tallant EA, Brosnihan KB, Diz DI: Counterregulatory actions of angiotensin-(1-7). *Hypertension* 1997; **30**: 535–541.
4. Loot AE, Roks AJ, Henning RH, *et al*: Angiotenisn-(1-7) attenuates the development of heart failure after myocardial infarction in rats. *Circulation* 2002; **105**: 1548–1550.
5. Brosnihihan KB, Li P, Ferrario CM: Angiotensin-(1-7) dilates canine coronary arteries through kinins and nitric oxide. *Hypertension* 1996; **27**: 523–528.
6. Roks AJM, van Geel PP, Pinto YM, *et al*: Angiotensin-(1-7) is a modulator of the human renin-angiotensin system. *Hypertension* 1999; **34**: 296–301.
7. Ueda S, Masumori-Maemoto S, Ashino K, *et al*: Angiotensin-(1-7) attenuates vasoconstriction evoked by angiotensin II but not by noradrenaline in man. *Hypertenison* 2000; **35**: 998–1001.
8. Gorelik G, Carbini LA, Scicli AG: Angiotensin-(1-7) induces bradykinin-mediated relaxation in porcine coronary artery. *J Pharmacol Exp Ther* 1998; **286**: 403–410.
9. Deddish PA, Marcic B, Jackman HL, Wang H-Z, Skidgel RA, Erdos EG: N-domain–specific substrate and C-domain inhibitors of angiotensin-converting enzyme: angiotenisn-(1-7) and keto-ACE. *Hypertension* 1998; **31**: 912–917.
10. Minshall RD, Tan F, Nakamura F, *et al*: Potentiation of the actions of bradykinin by angiotensin B2 recoptors and angiotensin-converting enzyme in CHO cells. *Circ Res* 1997; **81**: 848–856.
11. Benzing T, Fleming I, Blaukat A, Muller-Esterl W, Busse R: Angiotensin-converting enzyme inhibitor ramiprilat interferes with the sequenstration of the B2 kinin receptor within the plasma membrane of native endothelial cells. *Circulation* 1999; **99**: 2034–2040.
12. Danser AHJ, Tom B, de Vries R, Saxena PR: L-NAME–resistant bradykinin-induced relaxation in porcine coronary arteries is NO-dependent: effect of ACE inhibition. *Br J Pharmacol* 2000; **131**: 195–202.
13. Ichikawa S, Takayama Y: Long-term effects of olmesartan, an Ang II receptor antagonist, on blood pressure and the renin-angiotensin-aldosterone system in hypertensive patients. *Hypertens Res* 2001; **24**: 641–646.
14. Gavras I, Gavras H, Eprosartan Multinational Study Group: Effects of eprosartan *versus* enalapril in hypertensive patitents on the renin-angiotensin-aldosterone and safety parameters: results from a 26-week, double-blind, multicentre study. *Curr Med Res Opin* 1999; **15**: 15–24.
15. Grossman E, Peleg E, Carroll J, Shamiss A, Rosenthal T: Hemodynamic and humoral effects of the angiotensin II antagonist losartan in essentioal hypertension. *Am J Hypertens* 1994; **7**: 1041–1044.
16. Bouer IH, Reams GP, Wu Z, Lau-Sieckman A: Effects of losartan on the renin-angiotensin-aldosterone axis in essential hypertension. *J Hum Hypertens* 1995; **9**: 237–243.
17. Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM: Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. *Hypertension* 2004; **43**: 970–976.
18. Tom B, de Vries R, Saxena PR, Danser AHJ: Bradykinin potentiation by angiotensin-(1-7) and ACE inhibitors correlates with ACE C- and N-domain blockade. *Hypertension* 2001; **38**: 95–99.
19. Benter IF, Ferrario CM, Morris M, Diz DI: Antihypertensive actions of angiotensin-(1-7) in spontaneously hypertensive rats. *Am J Physiol* 1995; **269**: H313–H319.
20. Widdop RE, Sampey DB, Jarrott B: Cardiovascular effects of angiotenisn-(1-7) in conscious spontaneously hypertensive rats. *Hypertension* 1999; **34**: 964–968.
21. Kobayashi N, Nishikimi T, Horinaka S, Ishimitsu T, Matsuoka H: Effects of imidapril on NOS expression and myocardial remodeling in failing heart of Dahl-salt sensitive hypertensive rats. *Cardiovasc Res* 1999; **44**: 518–526.
22. Kobayashi N, Horinaka S, Mita S, *et al*: Aminoguanidine inhibits mitogen-activated protein kinase and improves cardiac performance and cardiovascular remodeling in failing hearts of salt-sensitive hypertensive rats. *J Hypertens* 2002; **20**: 2475–2485.
23. Turner AJ, Tipnis SR, Guy JL, Rice G, Hooper NM: ACEH/ACE2 is a novel mammalian metallocarboxypeptidase and a monologue of angiotensin-converting enzyme insensitive to ACE inhibitors. *Can J Physiol Pharmacol* 2002; **80**: 346–353.
24. Turner AJ, Hooper NM: The angiotensin-converting enzyme gene family: genomics and pharmacology. *Trends Pharmacol Sci* 2002; **23**: 177–183.
25. Crackower MA, Sarao R, Oudit GY, *et al*: Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 2002; **417**: 822–828.
26. Vickers C, Hales P, Kaushik V, *et al*: Hydrolysis of biological peptides by human angiotensin-converting enzyme–related carboxypeptidase. *J Biol Chem* 2002; **277**: 14838–14843.
27. Gallagher PE, Chappell MC, Bernish WB, Tallant EA: ACE2 expression in brain: angiotensin II down-regulates ACE2 in astrocytes. *Hypertension* 2003; **42**: 389.
28. Pool JL, Glazer R, Chiang YT, Gatlin M: Dose-response efficacy of valsartan, a new angiotensin II receptor blocker. *J Hum Hypertens* 1999; **13**: 275–281.
29. Fogari R, Mugellini A, Zoppi A, *et al*: Efficacy of losartan, valsartan, and telmisartan in patients with mild to moderate hypertension: a double-blind, placebo-controlled, crossover study using ambulatory blood pressure monitoring. *Curr Ther Res Clin Exp* 2002; **63**: 1–14.
30. Mendes AC, Ferreira AJ, Pinheiro SV, Santos RA: Chronic infusion of angiotensin-(1-7) reduces heart angiotensin II levels in rats. *Regul Pept* 2005; **125**: 29–34.
31. Walters PE, Gaspari TA, Widdop RE: Angiotensin-(1-7) acts as a vasodepressor agent *via* angiotensin II type 2 receptors in conscious rats. *Hypertension* 2005; **45**: 960–966.

32. Agata J, Chao L, Chao J: Kallikrein gene delivery improves cardiac reserve and attenuates remodeling after myocardial infarction. *Hypertension* 2002; **40**: 653–659.
33. Santos RA, Simoes e Silva AC, Maric C, *et al*: Angiotensin-(1-7) is an endogenous ligand for the G protein–coupled receptor Mas. *Proc Nat Acad Sci U S A* 2003; **100**: 8258–8263.
34. Taliant EA, Chappell MC, Ferrario CM, Gallagher PE: Inhibition of MAP kinase activity by angiotensin-(1-7) in vascular smooth muscle cells in mediated by the Mas receptor. *Hypertension* 2004; **43**: 1348 (Abstract).
35. Strawn WB, Ferrario CM, Tallant EA: Angiotensin-(1-7) reduces smooth muscle growth after vascular injury. *Hypertension* 1999; **33**: 207–211.
36. Ferreira AJ, Santos RAS, Almeida AP: Angiotensin-(1-7): cardioprotective effect in myocardial ischemia/reperfusion. *Hypertension* 2001; **38**: 665–668.
37. Averill DB, Ishiyama Y, Chappell MC, Ferrario CM: Cardiac angiotensin-(1-7) in ischemic cardiomyopathy. *Circulation* 2003; **108**: 2141–2146.