The present study examined the in vivo effects of candesartan cilexetil compared with losartan on angiotensin II (Ang II) receptor binding in the rat kidney after oral administration. Male Sprague-Dawley rats (250 to 300 g) were gavaged with candesartan cilexetil or losartan in doses of 0.1, 0.3, 1, 3, 10, or 30 mg/kg, or corresponding vehicle. Rats were killed at 0, 1, 2, 8, or 24 h after drug administration, trunk blood collected, and kidneys removed. The effects of candesartan cilexetil and losartan on Ang II receptor binding were determined by quantitative in vitro autoradiography using the radioligand \([^{125}\text{I}]\text{[Sar}^1\text{,Ile}^8\text{]}\text{Ang II}\). Ang II receptor binding in the kidney was mainly due to AT1 receptors with high levels of binding localized to the inner stripe of the outer medulla and glomeruli in cortical regions. Candesartan cilexetil (0.1 to 30 mg/kg) inhibited Ang II receptor binding to all anatomical sites of the kidney, in a dose-dependent manner. Losartan (0.1 to 30 mg/kg) also produced dose-dependent inhibition of Ang II receptor binding but was approximately 10- to 30-fold less potent than candesartan cilexetil. Inhibition of Ang II receptor binding was near maximal about 1 h after administration of candesartan cilexetil (10 mg/kg) or losartan (10 mg/kg), with both drugs producing persistent blockade at 24 h despite plasma renin activity and plasma drug concentrations returning to near normal levels. In vitro, candesartan, losartan, and EXP3174 \((1 \times 10^{-10} \text{ to } 1 \times 10^{-5} \text{ mol/L})\) displaced \([^{125}\text{I}]\text{[Sar}^1\text{,Ile}^8\text{]}\text{Ang II}\) binding from AT1 receptors in the kidney in a concentration-dependent manner with a rank order of potency of candesartan > EXP3174 > losartan. The concentration required to displace 50% of radioligand binding (\(IC_{50}\)) by candesartan, EXP3174, and losartan was 0.9 ± 0.1 nmol/L, 3.4 ± 0.4 nmol/L, and 8.9 ± 1.1 nmol/L, respectively. In conclusion, the findings of the present study suggest that candesartan cilexetil is more potent than losartan in antagonizing AT1 receptors in the kidney in vivo. Nonetheless, both candesartan cilexetil and losartan produce rapid, complete, and sustained blockade of AT1 receptors in the rat kidney. Tissue blockade of Ang II receptors in target organs, such as the kidney, may contribute to the beneficial effects of Ang II receptor antagonists as antihypertensive agents.  

**KEY WORDS:** Angiotensin receptors, AT1 receptors, angiotensin antagonists, AT1 receptor antagonists, candesartan, losartan.
The renin-angiotensin system (RAS) plays an important role in blood pressure regulation and cardiovascular function. Angiotensin II (Ang II) is the principal effector peptide of the RAS and has been implicated in the pathophysiology of hypertension and other cardiovascular disorders. Ang II mediates its actions by binding and activating specific Ang II receptors. To date, at least two major subtypes of Ang II receptors have been identified and cloned, namely AT₁ and AT₂. Virtually all the well-known cardiovascular actions of Ang II such as vasoconstriction, facilitation of sympathetic transmission, stimulation of cell growth, and aldosterone release are mediated by the AT₁ receptor. The functional role of the AT₂ receptor is still poorly understood but recent studies suggest that this receptor subtype may be involved in antiproliferation, apoptosis, differentiation, and, possibly, vasodilatation.

Ang II antagonists are a new class of drugs for the treatment of hypertension that interfere with the RAS by selectively blocking the AT₁ receptor subtype. Because most of the cardiovascular effects of Ang II are mediated by the AT₁ receptor, Ang II antagonists should provide more specific and complete blockade of the RAS. Although angiotensin-converting enzyme (ACE) inhibitors have proved a major advance in the treatment of hypertension and related disorders, these agents still have certain limitations that may restrict their therapeutic utility. In addition to inhibiting the formation of Ang II, ACE inhibitors also interfere with the metabolism of other peptides such as bradykinin and substance P, which is thought to be responsible for the common adverse effect of dry cough and the rare but life-threatening condition of angioedema. Moreover, Ang II can be formed by alternative pathways that are independent of ACE such that blockade of the RAS with ACE inhibitors is incomplete.

Candesartan cilexetil is a new, orally active, selective Ang II AT₁ receptor antagonist. This agent is a pro-drug that is hydrolyzed completely to the active compound candesartan during gastrointestinal absorption. In vitro studies have shown that candesartan displays high affinity for the AT₁ receptor and acts as a non-competitive antagonist. Losartan was the first Ang II AT₁ antagonist developed for the treatment of hypertension. Losartan per se is a competitive antagonist; however, it is converted in vivo to the potent active metabolite EXP3174, which displays non-competitive antagonism. As EXP3174 is more potent and has a longer half-life than the parent drug losartan, it is largely responsible for the actions of losartan in vivo.

There are pharmacologic differences between candesartan cilexetil and losartan, (ie, non-competitive versus competitive antagonism), which may affect their ability to effectively antagonize AT₁ receptors in vivo and, possibly therefore, their efficacy as antihypertensive agents. Although there are many in vitro data on the pharmacologic profiles of candesartan and losartan individually, few studies have directly examined the effects of these agents on receptor binding in vivo after oral administration. Quantitative in vitro autoradiography is a useful technique, which enables the localization and quantification of Ang II receptors in intact organs. This approach had been used successfully in the past to map the localization of Ang II receptors in a variety of tissues. The kidney is a major organ involved in cardiovascular homeostasis and represents a target tissue in antihypertensive therapy. The present study was therefore undertaken to compare the in vivo effects of candesartan cilexetil with losartan on Ang II receptor binding in the rat kidney after oral administration using quantitative in vitro autoradiography.

MATERIALS AND METHODS

Animals The experimental protocol was approved by the Austin & Repatriation Medical Centre Animal Ethics Committee and complied with the National Health and Medical Research Council of Australia guidelines for animal experimentation. Adult male Sprague-Dawley rats (250 –300 g) were housed at 25°C in a 12 h/12 h light/dark cycle with access to food and water ad libitum before experimentation. To study the dose response and time-course of Ang II receptor inhibition by candesartan cilexetil and losartan, four to five rats were randomly allocated to each dose level or time point.

Drug Administration Candesartan cilexetil and losartan were freshly prepared in 5% arabic gum and distilled water, respectively. In dose-response studies, rats were gavaged with candesartan cilexetil or losartan at doses of 0.1, 0.3, 1, 3, 10, and 30 mg/kg or corresponding vehicle and killed 1 h after administration. In time-course studies, rats were gavaged with candesartan cilexetil (10 mg/kg) or losartan (10 mg/kg) and killed at 0, 1, 2, 8, or 24 h after drug administration.

Tissue Preparation After drug administration, rats were killed by decapitation and exsanguinated. Trunk blood was collected into pre-chilled tubes containing heparin or EDTA for the determination of plasma drug concentration and plasma renin activity, respectively (see below). The kidneys were quickly removed, snap-frozen in isopentane-dry ice (−40°C), and stored at −80°C. Tissue sections of 20 µm thickness were cut on a cryostat (Microm HM 505E, Walldorf, Germany) at −20°C, thaw-mounted onto silane-coated slides, dehydrated overnight under reduced pressure at 4°C, and then stored at −80°C in sealed containers with silica gel until required.
Quantitative Autoradiography  Ang II receptor binding was determined by the technique of in vitro autoradiography using the radioligand $^{125}$I-[Sar$^1$,Ile$^8$] Ang II.$^{21-23}$ Briefly, slide-mounted tissue sections (20 μm) were incubated with sodium phosphate buffer containing 10 mmol/L Na$_2$HPO$_4$, 150 mmol/L NaCl, 5 mmol/L EDTA, 0.02% NaNO$_3$, 0.2% bovine serum albumin (BSA), 0.4 mmol/L bacitracin, pH 7.4, and 0.2 μCi/mL $^{125}$I-[Sar$^1$,Ile$^8$] Ang II for 1 h at room temperature. Non-specific binding was determined in parallel incubations in the presence of an excess (1 μmol/L) of unlabelled Ang II amide. After incubation, tissue sections were transferred through four successive 1-min washes of ice-cold buffer without BSA to remove non-specifically bound radioligand. The sections were dried under a stream of cold air, loaded into X-ray cassettes together with a set of radioactivity standards, and exposed to AgfaScopix CR3B X-ray film for 1 week at room temperature. After exposure, the films were developed and autoradiographs quantified using a microcomputer imaging device (MCID) analysis system (Imaging Research Inc., Toronto, Ontario, Canada). The radioactive standards were fitted to calibration curves and the optical density value of each pixel of digitized image converted into dpm/mm$^2$. Specific binding was then calculated by subtracting non-specific binding from total binding. The effects of candesartan cilexetil and losartan on Ang II receptor binding were quantitated and expressed as a percentage of tissue binding from control (vehicle-treated) rats. Some sections were stained with hematoxylin and eosin and examined with autoradiographs for the anatomical localization of $^{125}$I-[Sar$^1$,Ile$^8$] Ang II binding.

Plasma Renin Activity  Blood was collected into heparinized tubes containing 10 mmol/L EDTA on ice and subsequently centrifuged (Beckman GPR Centrifuge, Beckman Instruments, Palo Alto, CA) at 4°C for 10 min at 3500 rpm. The plasma was collected and stored at −80°C for later determination of plasma renin activity by radioimmunoassay, as described previously.$^{24}$

Plasma Drug Concentration  Plasma drug concentrations of either candesartan or losartan were determined by a radioreceptor binding displacement assay using rat liver membranes.$^{22,23,25}$ Briefly, untreated Sprague-Dawley rats were killed by decapitation and the liver dissected free. The liver was diced in small pieces and subsequently homogenized by a polytron homogenizer (Ultra-Turrax T-25, Janke and Kunkel, Staufen, Germany) in a buffer containing 0.25 mol/L sucrose, 20 mmol/L HEPES, 1 mmol/L EGTA, pH 7.4, including protease inhibitors (5 μg/mL leupeptin, 5 μg/mL antipain) and phenylmethylsulphonyl fluoride (10 mmol/L). The homogenate was centrifuged at 4°C for 5 min at 1500 rpm to remove all debris and nuclei. The pellet was discarded and the low-speed supernatant re-centrifuged at 4°C for 20 min at 14,000 rpm. The resulting pellet was resuspended in 5 mL ice-cold resuspension buffer containing 0.25 mol/L sucrose, 20 mmol/L HEPES, 0.5 mg/mL bacitracin, and stored at −150°C in liquid nitrogen until use. Liver membranes (500 μg protein/tube) were incubated with $^{125}$I-[Sar$^1$,Ile$^8$] Ang II (0.1 μCi/mL) at 25°C for 1 h in a total volume of 0.25 mL of sodium phosphate buffer (150 mmol/L NaCl, 10 mmol/L Na$_2$HPO$_4$, 5 mmol/L EDTA, pH 7.4). A standard curve was constructed by the addition of increasing concentrations of the AT$_1$ receptor antagonist, either candesartan or EXP3174. Unknown rat plasma samples were diluted 1:2 and added in place of the known standards. The reaction was terminated by the addition of 2.5 mL ice-cold sodium phosphate buffer. Bound and free ligand were separated by filtration through Whatman GF/C glass fiber filters using a Brandel automatic filtration apparatus (Biomedical Research and Development Laboratories Inc., Gathersburg, MD). Total bound radioactivity was measured by a gamma counter (LKB Wallac 1260 Multigamma II, Wallac, Finland). Non-specific binding was determined in the presence of 1 μmol/L unlabelled Ang II amide. Specific binding was subsequently calculated by subtracting non-specific binding from total binding. Displacement of radioligand binding by the unknown rat plasma samples was then compared with the standard displacement curve to the respective drug to estimate plasma concentrations of candesartan and losartan/EXP3174. In preliminary experiments, the AT$_2$ receptor antagonist PD123319 failed to displace radioligand binding in this assay system, which is consistent with the liver containing only AT$_1$ receptors.

Radioligand Binding Displacement Studies  Membrane Preparation  Renal membranes for radioligand binding studies were prepared as described previously.$^{26}$ Briefly, untreated Sprague-Dawley rats were killed by decapitation and the kidneys removed. The medullary region of the kidney was dissected free, diced, and then homogenized with a polytron homogenizer in 10 mL ice-cold buffer containing 0.25 mol/L sucrose, 20 mmol/L HEPES, 1 mmol/L EGTA, pH 7.4, including protease inhibitors (5 μg/mL leupeptin, 5 μg/mL antipain) and phenylmethylsulphonyl fluoride (10 mmol/L). The homogenate was centrifuged at 4°C for 5 min at 1500 rpm to remove all debris and nuclei. The pellet was discarded and the low-speed supernatant re-centrifuged at 4°C for 20 min at 14,000 rpm. The resulting pellet was resuspended in 5 mL ice-cold resuspension buffer containing 0.25 mol/L sucrose, 20 mmol/L HEPES, 0.5 mg/mL bacitracin, and stored at −150°C in liquid nitrogen until use.
and 1 mL aprotinin, and the re-suspension centrifuged at 14,000 rpm for 20 min. The supernatant was discarded and the final pellet re-suspended in 5 mL ice-cold re-suspension buffer and stored at −150°C in liquid nitrogen until use. Protein concentration was measured by the Bradford assay, using BSA as a standard.27,28

**In Vitro Displacement** Renal membranes (500 μg protein/tube) were incubated with [125I]-[Sar1,Ile8] Ang II (0.1 μCi/mL) at 25°C for 1 h in a total volume of 0.25 mL of sodium phosphate buffer (150 mmol/L NaCl, 10 mmol/L Na2HPO4, 5 mmol/L EDTA, pH 7.4). Displacement of [125I]-[Sar1,Ile8] Ang II binding from renal membranes in vitro by candesartan, losartan, and EXP3174 was determined by co-incubation with different concentrations of the AT1 receptor antagonists (1 × 10−10 to 1 × 10−5 mol/L). The reaction was terminated by the addition of 2.5 mL ice-cold sodium phosphate buffer. Bound and free ligand were separated by filtration through Whatman GF/C glass fiber filters using a Brandel automatic filtration apparatus and total bound radioactivity was measured by a gamma counter. Non-specific binding was determined in the presence of 1 μmol/L unlabelled Ang II amide. Specific binding was calculated by subtracting non-specific binding from total binding.

**Drugs and Materials** The antagonist analogue [Sar1,Ile8] Ang II was radioiodinated using the chloramine T method and purified by high-performance liquid chromatography.23 Candesartan cilexetil (TCV-116) and candesartan (CV-11974) were gifts from Asträ Håssle AB (Mölndal, Sweden). Losartan and EXP3174 were kindly provided by Merck, Sharp & Dohme (Sydney, NSW, Australia). [Sar1,Ile8] Ang II and Ang II amide were obtained from Peninsula Laboratories (Belmont, CA). All other drugs and reagents were purchased from Sigma (St. Louis, MO) or BDH (Poole, England, UK).

**Statistical Analysis** Results are expressed as mean ± standard error of the mean (SEM); n represents the number of rats. The data were analyzed by one-way or two-way analysis of variance (ANOVA) followed by Dunnett’s test or Student-Newman-Keuls (SNK) test, where appropriate. All statistical analyses were performed using the statistical program SigmaStat for Windows (Jandel Corporation, San Rafael, CA). In all cases, P < .05 was taken to indicate statistical significance.

**RESULTS**

**Effects of Candesartan Cilexetil and Losartan on Plasma Renin Activity** Oral administration of candesartan cilexetil (0.1–30 mg/kg) and losartan (0.1–30 mg/kg) produced a dose-dependent rise in plasma renin activity, with a maximal effect at 10 mg/kg and 30 mg/kg, respectively, 1 h after dosing (P < .05, ANOVA, Dunnett’s test) (Fig. 1A). The rise in plasma renin activity after administration of candesartan cilexetil (10 mg/kg) or losartan (10 mg/kg) peaked at 1 h, was maintained at 2 h and 8 h, and fell substantially at 24 h (P < .05, ANOVA, Dunnett’s test) (Fig. 1B). At most comparable doses, the effect of candesartan cilexetil on plasma renin activity was significantly greater than losartan (P < .05, ANOVA, SNK test).

**Plasma Drug Concentrations of Candesartan and Losartan** After drug administration (0.1–30 mg/kg, 1 h), plasma concentrations of candesartan and losartan rose steadily, in a dose-dependent fashion (P < .05, ANOVA, Dunnett’s test) (Fig. 1C). For a 10-mg/kg dose, plasma drug concentrations of candesartan and losartan peaked at approximately 1–2 h, were maintained at 8 h, and fell sharply at 24 h (P < .05, ANOVA, Dunnett’s test) (Fig. 1D). After oral administration, the rise in plasma losartan levels was slower but higher than plasma candesartan levels.

**Ang II Receptors in the Kidney** Fig. 2 shows computer-generated color autoradiographs of Ang II re-
ceptor binding in the rat kidney with the radioligand $[^{125}\text{I}]-[\text{Sar}^1,\text{Ile}^8]\text{Ang II}$. In control (vehicle-treated rats), Ang II receptor binding in the kidney was discrete, with high levels of binding localized to the inner stripe of the outer medulla and kidney cortex (Fig. 2A). In cortical regions, there was a high degree of punctate binding associated with glomeruli. In other experiments, Ang II receptor binding in the rat kidney was shown to be mostly due to AT$_1$ receptors. An excess of the AT$_1$ antagonist losartan (10 $\mu$mol/L) abolished Ang II receptor binding whereas an excess of the AT$_2$ antagonist PD123319 (10 $\mu$mol/L) was without effect (data not shown). Non-specific binding, as determined by the presence of an excess unlabelled Ang II amide (1 $\mu$mol/L), was very low and virtually undetectable (data not shown).

**In Vivo Effects of Candesartan Cilexetil and Losartan on AT$_1$ Receptors in the Kidney**
Fig. 2 (A–D) shows color-coded autoradiographs of kidney sections from rats pretreated with candesartan cilexetil. As can be seen, candesartan cilexetil (0.1, 0.3, and 1 mg/kg) inhibited Ang II receptor binding to all anatomical sites of the rat kidney 1 h after oral administration (Fig. 2A–D). As shown quantitatively in Fig. 3, candesartan cilexetil (0.1–30 mg/kg) inhibited Ang II receptor binding in the glomerulus (Fig. 3A) and inner stripe (Fig. 3C) of the kidney, in a dose-dependent manner ($P < .05$, ANOVA, Dunnett’s test). Losartan (0.1–30 mg/kg) also produced dose-dependent inhibition of Ang II receptor binding ($P < .05$, ANOVA, Dunnett’s test) but was significantly less potent (approximately 10- to 30-fold) than candesartan cilexetil ($P < .05$, ANOVA, SNK test) (Fig. 3A, C). Inhibition of Ang II receptor binding was maximal approximately 1–2 h after administration of candesartan cilexetil (10 mg/kg) or losartan (10 mg/kg), with both drugs producing persistent blockade at 8 and 24 h ($P < .05$, ANOVA, Dunnett’s test) (Fig. 3B, D). There was no
significant difference in the time-course of Ang II receptor inhibition between candesartan cilexetil (10 mg/kg) and losartan (10 mg/kg) (P < .05, ANOVA, SNK test).

**In Vitro Inhibition of AT₁ Receptor Binding in Renal Membranes**  
In radioligand binding studies, candesartan, losartan and EXP3174 (1 × 10⁻¹⁰ to 1 × 10⁻⁵ mol/L) in vitro displaced [¹²⁵I]-[Sar¹,Ile⁸] Ang II from renal membranes in a concentration-dependent manner (Fig. 4). The rank order of potency for inhibition of [¹²⁵I]-[Sar¹,Ile⁸] Ang II binding was candesartan > EXP3174 > losartan. The concentration of candesartan, EXP3174, and losartan required to displace 50% of radioligand binding (IC₅₀) was 0.9 ± 0.1 nmol/L, 3.4 ± 0.4 nmol/L, and 8.9 ± 1.1 nmol/L, respectively.

**DISCUSSION**

Although much is known about the binding characteristics of candesartan in vitro, few studies have examined the inhibition of Ang II receptor binding by this agent in vivo, particularly after oral administration. Pharmacokinetic and pharmacodynamic differences exist between the different members of the Ang II receptor antagonist class, which may potentially influence the nature of Ang II receptor inhibition in vivo. The present study was performed to study the dose-response and time-course effects of candesartan cilexetil on tissue Ang II receptor binding in vivo after oral administration, in comparison with the prototype drug losartan. The in vivo effects of these AT₁ receptor antagonists on Ang II receptor binding in the rat kidney were examined by the unique and established technique of quantitative in vitro autoradiography using the specific Ang II receptor radioligand [¹²⁵I]-[Sar¹,Ile⁸] Ang II.²¹–²³ Consistent with previous reports, Ang II receptor binding in the rat kidney was discrete, with high levels of binding localized to the inner stripe of the outer medulla and the cortex. In cortical regions, a high degree of punctate binding was evident, which is associated with glomeruli. Ang II receptors in the rat kidney were predominantly of the AT₁ receptor subtype, as determined by their differential sensitivity to AT₁- and AT₂-selective antagonists, losartan and PD123319, respectively.

This study provides evidence that candesartan cilexetil in vivo is a highly potent AT₁ receptor antagonist. Candesartan cilexetil (0.1–30 mg/kg) inhibited Ang II receptor binding to all anatomical sites of the rat kidney in a dose-dependent manner. At a dose of 1 mg/kg, candesartan cilexetil virtually abolished Ang II receptor binding to all sites in the kidney. Losartan also caused dose-dependent inhibition of Ang II receptor binding but was at least 10- to 30-fold less potent than candesartan cilexetil. However, the time-course of AT₁ receptor inhibition for both candesartan

![FIG. 3. In vivo effects of candesartan cilexetil and losartan on Ang II receptor binding in the glomerulus (A, B) and inner stripe (C, D) of the rat kidney after oral administration. Dose-response relationship (0.1–30 mg/kg) after 1 h (A, C) and time-course relationship (0–24 h) for 10 mg/kg dose (B, D). Data are expressed as a percentage of specific binding from vehicle-treated rats (% control). Each column represents the mean ± SEM; n = 4–5 rats per group.](https://academic.oup.com/ajh/article-abstract/13/9/1005/256584/1010-Fabiani-et-al)

![FIG. 4. In vitro displacement of specific binding of the radioligand [¹²⁵I]-[Sar¹,Ile⁸] Ang II from kidney membranes by increasing concentrations (1 × 10⁻¹⁰ to 1 × 10⁻⁵ mol/L) of candesartan, losartan, and EXP3174. Data are expressed as a percentage of specific binding in the absence of any drugs (% control). Each point represents the mean ± SEM; n = 3 experiments performed in triplicate.](https://academic.oup.com/ajh/article-abstract/13/9/1005/256584/1010-Fabiani-et-al)
Candesartan cilexetil and losartan did not differ. Inhibition of Ang II receptor binding in the rat kidney was maximal approximately 1–2 h after oral administration of either candesartan cilexetil or losartan, with both drugs producing persistent receptor inhibition for up to 24 h, despite plasma renin activity and plasma drug levels for each drug returning to near normal levels (see below). This suggests that oral dosing with candesartan cilexetil or losartan provides effective and sustained inhibition of AT1 receptors in target tissues such as the kidney over a 24-h period.

The increased potency of candesartan cilexetil over losartan in vivo is apparently due to the high affinity of the active compound candesartan for AT1 receptors. This was demonstrated in radioligand displacement studies in vitro, in which candesartan was more potent than losartan or its active metabolite EXP3174 in displacing $^\text{[125I]}$-[Sar1,Ile8] Ang II binding from AT1 receptors on rat renal membranes. The rank order of potency for inhibition of $^\text{[125I]}$-[Sar1,Ile8] Ang II binding from renal membranes was candesartan > EXP3174 > losartan. These findings in the rat kidney complement previous work in which candesartan in vitro displayed very high affinity in displacing specific $^\text{[125I]}$-[Sar1,Ile8] Ang II binding from AT1 receptors in rabbit aorta and bovine adrenal cortex. 29–31

Our in vivo binding data with candesartan cilexetil and losartan correlate well with functional studies in vivo. Candesartan cilexetil, at a dose of 0.3 mg/kg, has been shown to totally inhibit the pressor response to Ang II in rats, which was still pronounced after 24 h. 30 In contrast, losartan was only effective in inhibiting Ang II-induced pressor responses at doses greater than or equal to 3 mg/kg. 30 Candesartan cilexetil has also been shown to exert potent and long-lasting blood-pressure-lowering effects in several animal models of hypertension. 31,32 In acute studies, candesartan cilexetil effectively reduced blood pressure in spontaneously hypertensive rats (SHR), which still persisted after 24 h. Losartan also produced similar reductions in blood pressure in SHR but only at (~10-fold) higher doses. Furthermore, repeated oral administration of candesartan cilexetil (1 mg/kg) once daily for 2 weeks to SHR markedly reduced blood pressure for more than 24 h. Candesartan cilexetil also had sustained antihypertensive effects in two-kidney, one-clip (2K,1C) and one-kidney, one-clip (1K,1C) hypertensive rats and was significantly more potent than losartan. 31,32 Taken together, these findings suggest that candesartan cilexetil in vivo is more potent than losartan in blocking AT1 receptors and mediating its antihypertensive effects.

The higher potency of candesartan cilexetil may possibly reflect its potential ability clinically to reduce elevated blood pressure to a greater extent than losartan. In mild-moderate hypertension, 8 or 16 mg candesartan cilexetil was more effective than 50 mg losartan once daily in lowering blood pressure, 24 h after oral administration. 33,34 However, it should be emphasized that in this study the dosages may not have been therapeutically equivalent. More recently, though, candesartan cilexetil (16 mg) was reported to produce a significantly greater fall in blood pressure than the higher dose of losartan (100 mg) at 8 weeks in patients with mild-moderate hypertension. 35 Interestingly, after a missed dose, the antihypertensive effect of candesartan cilexetil (16 mg) was maintained whereas systolic and diastolic blood pressure returned towards baseline with losartan (100 mg). 35

As expected, AT1 receptor blockade by both candesartan cilexetil and losartan increased plasma renin activity. This reactive rise in plasma renin activity by candesartan cilexetil and losartan is presumably due to interruption of AT1 receptor-mediated negative-feedback of renin release. In general, the effects of candesartan cilexetil and losartan on plasma renin activity correlated with their plasma drug levels. It should be noted, though, that the facilitative effect on plasma renin activity by candesartan cilexetil was significantly greater than that of losartan, although plasma levels of candesartan cilexetil were generally lower than those of losartan and its metabolites. On the other hand, the slow but higher rise in plasma losartan levels suggests that conversion is slower but more complete in the rat. The facilitative effect of candesartan cilexetil on plasma renin activity is again probably related to the higher potency and affinity of candesartan cilexetil. AT1 receptors or possibly to better tissue bioavailability or access. In time-course studies, it is unclear why plasma renin activity returns to near normal levels after 24 h while there is still persistent inhibition of kidney AT1 receptors, given that the rise in plasma renin activity is also presumably due to blockade of AT1 receptor-mediated inhibition of renin release.

In summary, we have demonstrated the time-course and dose-response of inhibition of Ang II receptor binding in vivo after oral administration of candesartan cilexetil in comparison to the prototype losartan. The findings of the present study suggest that candesartan cilexetil is more potent than losartan in antagonizing AT1 receptors in the kidney in vivo. Nonetheless, both candesartan cilexetil and losartan produce rapid, complete, and sustained blockade of AT1 receptors in the rat kidney. Tissue blockade of Ang II receptors in target organs, such as the kidney, may contribute to the beneficial effects of Ang II receptor antagonists as antihypertensive agents.

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REFERENCES


