Comparative in vivo effects of irbesartan and losartan on angiotensin II receptor binding in the rat kidney following oral administration

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ABSTRACT

We examined the ability of the new non-peptide angiotensin II receptor antagonist irbesartan to inhibit AT₁ receptors in vivo in the rat kidney following oral administration, compared with the prototype drug losartan. Male Sprague–Dawley rats (250–300 g) were gavaged with either irbesartan or losartan at doses of 1, 3, 10, 30 or 100 mg/kg, or with corresponding vehicle. Rats were killed at 0, 1, 2, 8, or 24 h after drug administration, trunk blood was collected and the kidneys were removed. The effects of irbesartan and losartan on angiotensin II receptor binding were determined by quantitative in vitro autoradiography using the specific radioligand 125I-[Sar¹,Ile⁸]angiotensin II. High levels of angiotensin II receptor binding in the rat kidney were demonstrated in the glomeruli and inner stripe of the outer medulla, which was attributed to AT₁ receptors. At 1 h after dosing, irbesartan (1–100 mg/kg) and losartan (1–30 mg/kg) significantly inhibited AT₁ receptor binding in all anatomical areas of the kidney, in a dose-dependent manner, with a maximal effect at 100 mg/kg and 30 mg/kg respectively. For a 10 mg/kg dose, inhibition of AT₁ receptor binding was maximal around 1–2 h after oral administration of losartan, whereas maximal binding occurred between 2 and 8 h for irbesartan; both drugs produced persistent tissue blockade at 24 h. In radioligand binding studies, irbesartan, losartan and EXP3174 (1 × 10⁻¹⁰ to 1 × 10⁻⁵ M) displaced 125I-[Sar¹,Ile⁸]angiotensin II binding from renal AT₁ receptors in a concentration-dependent manner, with a rank order of potency of irbesartan > EXP3174 > losartan. The concentration required to displace 50% of radioligand binding (IC₅₀) by irbesartan, EXP3174 and losartan was 1.00 ± 0.2 nM, 3.5 ± 0.4 nM and 8.9 ± 1.1 nM respectively. In conclusion, the findings of the present study suggest that irbesartan and losartan produce effective and sustained inhibition of AT₁ receptors in vivo in the kidney following oral administration. However, irbesartan appears less potent, with respect to dosage, than losartan in vivo, despite having a higher affinity for AT₁ receptors in vitro. The reason for this apparent discrepancy is unclear, but it may reflect the slower onset of action of irbesartan and its rate of tissue accessibility. Inhibition of angiotensin II receptors in target tissues such as the kidney may represent an important action of AT₁ receptor antagonists, which may contribute to the beneficial effects of these agents in the clinical setting.

INTRODUCTION

The renin–angiotensin system (RAS) is a key hormonal system that is instrumental in the regulation of blood pressure and cardiovascular homeostasis [1,2]. The effects of the RAS that serve to restore blood pressure are mediated by the potent effector peptide angiotensin II. Such actions of angiotensin II include vasoconstriction,

Key words: angiotensin, angiotensin II antagonists, angiotensin receptors, AT₁ receptors, AT₂ receptor antagonists, irbesartan, losartan.

Abbreviations: ACE, angiotensin-converting enzyme; RAS, renin–angiotensin system.

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stimulation of sodium and fluid reabsorption, facilitation of sympathetic transmission and stimulation of cell growth [2,3]. As such, hyperactivity of the RAS, resulting in excess production of angiotensin II, has been linked directly to the development of hypertension and other forms of cardiovascular disease [4,5]. The actions of angiotensin II are mediated by at least two receptor subtypes, denoted AT_1_ and AT_2_ [6,7]. All the classical effects of angiotensin II, as well as the AT_1_ receptor. Much less is known about the role of the AT_2_ receptor, but recent evidence suggests that it mediate the effects mediated by the AT_1_ receptor [8,9]. Thus the AT_2_ receptor may play a role in mediating inhibition of cell growth, apoptosis and vasodilatation.

The critical role of the RAS in the pathophysiology of cardiovascular disease is highlighted by the tremendous success of angiotensin-converting enzyme (ACE) inhibitors in the treatment of hypertension and congestive heart failure [5,10,11]. ACE inhibitors, such as captopril, perindopril and enalapril, interrupt the RAS by inhibiting the synthesis of angiotensin II. However, given that ACE is not a specific enzyme, ACE inhibitors are known to interfere with the breakdown of bradykinin, substance P and other peptides [12]. Accumulation of these peptide substrates may be responsible for the common side-effect of dry cough and the rare but serious angioedema associated with the use of ACE inhibitors [13]. Furthermore, alternative non-ACE pathways are known to exist that can also generate angiotensin II, such that ACE inhibitors may not totally suppress angiotensin II production [14,15]. Moreover, ACE inhibitors provide competitive blockade, such that increasing levels of the peptide substrate angiotensin I, which would arise following chronic treatment, may actually displace the ACE inhibitor from the enzyme itself [16]. Indeed, it is well known that plasma ACE activity returns to normal pre-drug levels after prolonged administration of ACE inhibitors [16].

Angiotensin II receptor antagonists represent a new advance in the treatment of hypertension [17,18]. These agents provide more specific and complete blockade of the RAS by selectively antagonizing the AT_1_ receptor, and thus prevent the detrimental actions of angiotensin II at its site of action, regardless of how the peptide is generated. The enhanced specificity of action of AT_1_ receptor antagonists may help to overcome limitations imposed by ACE inhibitors. Theoretically, the beneficial effects of angiotensin II receptor blockers may relate not only to the blockade of AT_1_ receptors, but also to stimulation of unopposed AT_2_ receptors.

Irbesartan (SR 47436; BMS 186295) is a new long-acting non-peptide insurmountable angiotensin II AT_1_ receptor antagonist developed as an anti-hypertensive agent for clinical use, and is perhaps the best characterized [23,24]. Although the parent drug losartan itself is functionally active as a competitive antagonist, it is converted into the more potent and longer-acting active metabolite EXP3174, which displays non-competitive properties and, hence, is mainly responsible for its in vivo effects [25,26].

In binding studies, irbesartan has been shown to have high affinity for the AT_1_ receptor in rat liver membranes and human aortic smooth muscle cells in vitro, and was approximately 10-fold more potent than losartan [27,28]. Moreover, irbesartan displayed > 10 000-fold greater specificity for the AT_1_ receptor compared with the AT_2_ receptor, and did not display any activity towards various other receptors, ion channels, renin or ACE [27]. In functional studies, irbesartan in vitro has been demonstrated to produce concentrations-dependent and insurmountable antagonism of the contractile response to angiotensin II in rabbit aorta [27,29]. Thus irbesartan caused a rightward shift in the concentration-response curve to angiotensin II and depression of the maximum response. Moreover, irbesartan has been shown to inhibit in vivo the pressor response to angiotensin II in pithed rats in a non-competitive manner [30].

The kidney is an important organ involved in cardiovascular regulation and fluid/electrolyte dynamics, and thus is a key target in the treatment of hypertension. In the kidney, angiotensin receptors are localized predominantly to the renal vasculature, glomeruli and tubules, and are believed to modulate renal haemodynamics and sodium transport from the renal tubules [31–33].

The ability of angiotensin II receptor blockers to manifest their anti-hypertensive efficacy is dependent on how effectively they inhibit AT_1_ receptors in vivo in relevant cardiovascular tissues. Pharmacodynamic and/or pharmacokinetic differences between different angiotensin II receptor antagonists may therefore potentially impact on the efficacy of these agents. Although many studies have examined the pharmacological properties of either irbesartan or losartan, particularly in vitro, few studies have compared directly the effects of these drugs in vivo.

Quantitative in vitro autoradiography is a powerful technique that allows the anatomical localization of angiotensin II receptors and quantitative analysis in intact tissues. This technique has been used previously to localize angiotensin II receptors in various tissues and to study receptor inhibition by AT_1_ receptor antagonists [34–36]. To the best of our knowledge, the inhibitory effects of irbesartan on angiotensin II receptors have not been studied by quantitative in vitro autoradiography. Thus the present study was undertaken to examine the effects of irbesartan in vivo, compared with those of the prototype drug losartan, on angiotensin II receptor
binding in the rat kidney following oral administration using quantitative in vitro autoradiography.

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 (200–300 g) were housed at 25 °C in a 12 h/12 h light/dark cycle with access to food and water ad libitum, prior to experimentation.

Drug administration
Irbesartan and losartan were freshly prepared in 0.6% methylcellulose and distilled water respectively. In dose–response studies, rats were gavaged with either irbesartan or losartan at doses of 1, 3, 10, 30 or 100 mg/kg, or with corresponding vehicle, and killed 1 h after administration. In time-course studies, rats were gavaged with irbesartan (10 mg/kg) or losartan (10 mg/kg) and killed at 0, 1, 2, 8 or 24 h after drug administration.

Tissue preparation
Following 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/solid CO₂ (−40 °C) and stored at −80 °C. Tissue sections of 20 μm thickness were cut on a cryostat (HM 505E; Microm, Walldorf, Germany) at −20 °C, thaw-mounted on to 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
Angiotensin II receptor binding was determined by the technique of in vitro autoradiography using the radioligand [125]I-[Sar₁,Ile₈]angiotensin II [34–36]. Briefly, slide-mounted tissue sections (20 μm) were incubated with sodium phosphate buffer (10 mM Na₂HPO₄, 150 mM NaCl and 5 mM EDTA, pH 7.4) containing 0.02% NaN₃, 0.2% (w/v) BSA, 0.4 mM bacitracin and 0.2 μCi/ml [125]I-[Sar₁,Ile₈]angiotensin II for 1 h at room temperature. Non-specific binding was determined in parallel incubations in the presence of an excess (1 μM) of unlabelled angiotensin II amide. After incubation, tissue sections were transferred through four successive 1-min washes of ice-cold buffer without BSA in order 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 were quantified using a micro-computer imaging device (MCID) analysis system (Imaging Research Inc., Toronto, Ontario, Canada). The radioactive standards were fitted to calibration curves, and the absorbance value of each pixel of digitized image was converted into a value of d.p.m./mm². Specific binding was then calculated by subtracting non-specific binding from total binding. The effects of irbesartan and losartan on angiotensin II receptor binding were quantified and expressed as a percentage of tissue binding from control (vehicle-treated) rats. Some sections were stained with haematoxylin and eosin and examined with autoradiographs for the anatomical localization of [125]I-[Sar₁,Ile₈]angiotensin II binding.

Plasma renin activity
Blood was collected into heparinized tubes containing 10 mM EDTA on ice and subsequently centrifuged (J2-MC or GPR Centrifuge, Beckman Instruments, Palo Alto, CA, U.S.A.) at 4 °C for 10 min at 2740 g. The plasma was collected and stored at −80 °C for later determination of plasma renin activity by radioimmunoassay, as described previously [37].

Plasma drug concentrations
Plasma drug concentrations of either irbesartan or losartan were determined by a radioreceptor binding displacement assay using rat liver membranes [35,36,38]. Briefly, untreated Sprague–Dawley rats were killed by decapitation and the liver was dissected free. The liver was diced into small pieces and subsequently homogenized by a Polytron homogenizer (Ultra-Turrax T-25; Janke and Kunkel, Staufen, Germany) in a buffer containing 0.25 M sucrose, 20 mM Heps, 1 mM EDTA, pH 7.4, including protease inhibitors (5 μg/ml leupeptin, 5 μg/ml antipain and 10 mM PMSF). The homogenate was centrifuged at 4 °C for 15 min at 8740 g, the pellet discarded, and the supernatant re-centrifuged at 4 °C for 1 h at 48000 g. The supernatant was then discarded and the pellet was resuspended in resuspension buffer containing 0.25 M sucrose, 20 mM Heps, 0.5 mg/ml bacitracin and 100 units/ml aprotinin. The resuspension was centrifuged at 48000 g for 1 h. The final pellet was resuspended in the resuspension buffer and stored at −150 °C in liquid nitrogen until use.

Liver membranes (500 μg of protein/tube) were incubated with [125]I-[Sar₁,Ile₈]angiotensin II (0.1 μCi/ml) at 25 °C for 1 h in a total volume of 0.25 ml of sodium phosphate buffer (150 mM NaCl, 10 mM Na₂HPO₄, 5 mM EDTA, pH 7.4). A standard curve was constructed by the addition of increasing concentrations of the AT₁ receptor antagonist.
receptor antagonist (irbesartan, losartan or EXP3174). Rat plasma samples containing unknown drug concentrations were diluted 1:2 (v/v) and added in place of the known standards. The reaction was terminated by the addition of 2.5 ml of ice-cold sodium phosphate buffer. Bound and free ligand were separated by filtration through Whatman GF/C glass fibre filters using a Brandel automatic filtration apparatus (Biomedical Research and Development Laboratories Inc., Gaithersburg, MD, U.S.A.). Total bound radioactivity was measured in a γ-radiation counter (LKB Wallac 1260 Multigamma II). Non-specific binding was determined in the presence of 1 μM unlabelled angiotensin 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 of the respective drug to estimate plasma concentrations (ng/ml equivalent) of irbesartan and losartan/EXP3174.

It is important to note that this technique lacks some specificity, in that it cannot discriminate between the relative amounts of losartan and EXP3174, but rather measures total plasma losartan/EXP3174. In preliminary experiments, the AT₁ receptor antagonist PD123319 failed to displace radioligand binding in this assay system, which is consistent with the liver containing only AT₁ receptors.

**Radioligand binding displacement studies**
Renal membranes for radioligand binding studies were prepared as described previously [39,40]. 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 of ice-cold buffer (0.25 M sucrose, 20 mM Hepes, 1 mM EGTA, pH 7.4) containing protease inhibitors (5 μg/ml leupeptin, 5 μg/ml antipain and 10 mM PMSF). The homogenate was centrifuged at 4 °C for 5 min at 500 g to remove all debris and nuclei. The pellet was discarded and the low-speed supernatant was re-centrifuged at 4 °C for 20 min to 23700 g. The resulting pellet was resuspended in 5 ml of ice-cold resuspension buffer (0.25 M sucrose, 20 mM Hepes, 0.5 mg/ml bacitracin and 100 units/ml aprotinin) and the resuspension was centrifuged at 23700 g for 20 min. The supernatant was discarded and the final pellet was resuspended in 5 ml of ice-cold resuspension buffer and stored at −150 °C in liquid nitrogen until use. Protein concentration was measured by the Bradford assay, using BSA as a standard [41,42].

Renal membranes (500 μg of protein/tube) were incubated with 125I-[Sar₁,Ile₈]angiotensin II (0.1 μCi/ml) at 25 °C for 1 h in a total volume of 0.25 ml of sodium phosphate buffer (150 mM NaCl, 10 mM Na₂HPO₄, 5 mM EDTA, pH 7.4). Displacement of binding of 125I-[Sar₁,Ile₈]angiotensin II from renal membranes in vitro by irbesartan, losartan and EXP3174 was determined by co-incubation with various concentrations of the AT₁ receptor antagonists (1 × 10⁻¹⁰–1 × 10⁻⁵ M). The reaction was terminated by the addition of 2.5 ml of ice-cold sodium phosphate buffer. Bound and free ligand were separated by filtration through Whatman GF/C glass fibre filters using a Brandel automatic filtration apparatus, and total bound radioactivity was then measured by a γ-radiation counter. Non-specific binding was determined in the presence of 1 μM unlabelled angiotensin II amide. Specific binding was calculated by subtracting non-specific binding from total binding.

**Drugs and materials**
The antagonist analogue 125I-[Sar₁,Ile₈]angiotensin II was radio-iodinated using the chloramine T method and purified by HPLC [36]. Irbesartan was a gift from Bristol Myers Squibb (Princeton, NJ, U.S.A.). Losartan potassium and EXP3174 were kindly provided by Merck, Sharp & Dohme (Sydney, NSW, Australia). [Sar₁,Ile₈]angiotensin II and angiotensin II amide were obtained from Peninsula Laboratories (Belmont, CA, U.S.A.). All other drugs and reagents were purchased from Sigma (St Louis, MO, U.S.A.) or BDH (Poole, Dorset, U.K.).

**Statistical analysis**
Results are expressed as mean ± S.E.M.; n represents the number of rats. The data were analysed by one-way or two-way ANOVA followed by Dunnett’s test or Student–Newman–Keuls test, where appropriate. All statistical analyses were performed using the statistical program SigmaStat® for Windows (Jandel Corporation). In all cases, probability levels less than 0.05 (P < 0.05) were taken to indicate statistical significance.

**RESULTS**

**In vivo effects of irbesartan and losartan on angiotensin II receptor binding in kidney**
Figure 1 shows the autoradiographic localization of angiotensin II receptors (total binding) in the rat kidney using the radioligand 125I-[Sar₁,Ile₈]angiotensin II. As can be seen, angiotensin II receptor binding in the kidney was discrete and confined to the cortex and inner stripe of the outer medulla (Figure 1A). In cortical regions, there were high levels of punctate binding corresponding to glomeruli. Non-specific binding, determined in the presence of an excess of unlabelled angiotensin II amide (1 μM), was very low and similar to background (Figure 1B). The faint residual binding in the presence of unlabelled angiotensin II amide accounted for < 10% of total binding, with specific binding thus representing...
Figure 1  Autoradiographic localization of angiotensin II receptors in rat kidney (total binding) using the radioligand $^{125}$I-[Sar$^1$,Ile$^8$]angiotensin II

(A) Control (vehicle); (B) non-specific binding; (C)–(F) various times after oral administration of irbesartan (10 mg/kg): (C) 1 h, (D) 2 h, (E) 8 h and (F) 24 h. Red represents high levels of binding, yellow and green represent moderate levels of binding, and blue represents low levels of binding or background. Note that high levels of angiotensin II receptor binding were evident in the glomeruli and inner stripe of the kidney, which were inhibited by irbesartan.
Figure 2 Dose-related effects of irbesartan (1–100 mg/kg) and losartan (1–30 mg/kg) in vivo on angiotensin II receptor binding in the glomerulus (upper panel) and inner stripe (lower panel) of the rat kidney 1 h after oral administration. Data are expressed as a percentage of specific binding in kidneys from vehicle-treated rats (% of control). Each column represents the mean ± S.E.M. (n = 4–5 rats per group). nt, not tested.

In vitro effects of irbesartan and losartan on angiotensin II receptor binding in kidney membranes

In radioligand binding studies, irbesartan, losartan and EXP3174 (1×10⁻¹⁰–1×10⁻⁵ M) in vitro displaced [¹²⁵I][Sar¹,Ile⁸]angiotensin II binding from AT₁ receptors on rat renal membranes, in a concentration-dependent manner.
In vivo inhibition by irbesartan and losartan

Figure 4 In vitro inhibition of specific $^{125}$I-[Sar$^{1}$,Ile$^{8}$]angiotensin II binding to rat kidney membranes by increasing concentrations of irbesartan, losartan and EXP3174

The drug concentration range used was $1 \times 10^{-18}$ to $1 \times 10^{-11}$ M. Data are expressed as a percentage of specific binding in the absence of any drugs (% of control). Values represent means ± S.E.M. ($n = 3$ experiments performed in triplicate).

manner (Figure 4). The rank order of potency for inhibition of $^{125}$I-[Sar$^{1}$,Ile$^{8}$]angiotensin II binding was irbesartan > EXP3174 > losartan. The concentrations of irbesartan, EXP3174 and losartan required to displace 50% of radioligand binding (IC$_{50}$) were 1.0 ± 0.2 nM, 3.5 ± 0.4 nM and 8.9 ± 1.1 nM respectively.

Effects of irbesartan and losartan on plasma renin activity

Following oral administration, irbesartan (1–100 mg/kg) and losartan (1–30 mg/kg) produced a dose-dependent increase in plasma renin activity, with a maximal effect 1 h after dosing at 100 mg/kg and 30 mg/kg respectively ($P < 0.05$; ANOVA, Dunnett’s test) (Figure 5, upper panel). Plasma renin activity was significantly elevated at 1 and 2 h after oral administration of irbesartan (10 mg/kg) and losartan (10 mg/kg) ($P < 0.05$; ANOVA, Dunnett’s test) (Figure 5, lower panel). Plasma renin activity following administration of irbesartan (10 mg/kg) or losartan (10 mg/kg) continued to rise up to 8 h, but had fallen by 24 h ($P < 0.05$; ANOVA, Dunnett’s test) (Figure 5, lower panel).

Plasma drug concentrations of irbesartan and losartan

Following oral administration of irbesartan (1–100 mg/kg) and losartan (1–30 mg/kg), corresponding plasma drug concentrations rose in a dose-dependent manner ($P < 0.05$; ANOVA, Dunnett’s test) (Table 1). For a 10 mg/kg dose, plasma drug concentrations of irbesartan and losartan peaked at approx. 1–2 h and were still markedly elevated at 8 h ($P < 0.05$; ANOVA, Dunnett’s test) (Table 1). At 24 h after oral administration, the plasma drug concentration of losartan had fallen sharply, but that of irbesartan was still significantly elevated ($P < 0.05$; ANOVA and Dunnett’s or Student–Newman–Keuls test) (Table 1).

DISCUSSION

The present study was undertaken to examine the effects of irbesartan on angiotensin II receptor binding in vivo in the rat kidney following oral administration, as compared with those of the prototype AT$_1$ receptor antagonist losartan. The effects of these non-peptide agents on angiotensin II receptor binding in the rat kidney were examined by quantitative in vitro autoradiography using the radioligand $^{125}$I-[Sar$^{1}$,Ile$^{8}$]angiotensin II, a stable
Table 1  Plasma drug concentrations following oral administration of irbesartan and losartan

In the dose–response study (a), levels were measured 1 h after oral administration. In the time-course study (b), levels were measured after a 10 mg/kg oral dose. Values are means ± S.E.M.

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<th>Dose (mg/kg)</th>
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<td>1</td>
<td>125 ± 14</td>
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<td>579 ± 139</td>
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<tr>
<th>Time (h)</th>
<th>Irbesartan</th>
<th>Losartan</th>
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<td>0</td>
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<tr>
<td>1</td>
<td>369 ± 34</td>
<td>158 ± 43</td>
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<td>2</td>
<td>449 ± 45</td>
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<td>8</td>
<td>542 ± 134</td>
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<td>24</td>
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an analogue of angiotensin II. Quantitative in vitro autoradiography allows quantitative analysis of receptor binding in specific anatomical regions in whole intact tissues. In complex organs such as the kidney, in which angiotensin II receptors are localized to discrete anatomical areas, the technique of in vitro autoradiography allows precise determination of angiotensin II receptor inhibition in specific cellular structures without destroying tissue morphology. This technique has been used previously to localize angiotensin II receptors in numerous tissues and to study receptor inhibition by angiotensin II receptor antagonists [34–36].

In the present study, and consistent with previous accounts, angiotensin II receptor binding in the rat kidney was discretely localized to the cortex and inner stripe of the outer medulla. Angiotensin II receptor binding in the cortex was punctate, consistent with binding to glomeruli. Furthermore, angiotensin II receptors in the rat kidney are mainly of the AT₁ receptor subtype, in that the AT₁, antagonist losartan totally displaced angiotensin II receptor binding, whereas the AT₂ antagonist PD123319 was without effect.

Importantly, the findings from the present study provide direct evidence that irbesartan in vivo produces effective and sustained inhibition of angiotensin AT₁ receptors in the kidney, in a similar fashion to the prototype AT₁ receptor antagonist losartan. Both irbesartan and losartan inhibited angiotensin II receptor binding in all anatomical areas of the rat kidney, in a dose- and time-dependent manner.

Data from dose–response studies suggest that inhibition of angiotensin II receptor binding in the glomerulus and inner stripe of the outer kidney is maximal at doses of 100 mg/kg irbesartan and 30 mg/kg losartan at 1 h after oral administration. This finding may suggest that irbesartan is less potent than losartan in vivo at maximally inhibiting angiotensin II receptor binding in the kidney. However, this is at variance with our in vitro data, where irbesartan was shown to be more potent in displacing angiotensin II receptor binding from renal membranes, compared with losartan or its active metabolite EXP3174. This paradox is also evident in the clinical situation, in which the recommended oral dose range for irbesartan is 150–300 mg/day, as compared with that of losartan of 50–100 mg/day [17,19,24].

It should be emphasized that in vivo rat studies with irbesartan in the literature are very limited. Thus the findings of the present study have been mostly discussed with respect to existing human data, partly because of this deficiency, but more importantly to extrapolate our findings in the rat to the human scenario in order to derive clinical relevance.

The apparent lower potency of irbesartan compared with losartan in vivo does not appear to be due to a lower affinity of irbesartan for AT₁ receptors. This is evident from the radioligand displacement studies, in which irbesartan in vivo was more potent than losartan, or its active metabolite EXP3174, in displacing ¹²⁵I-[Sar¹,Ile₅] angiotensin II binding from AT₁ receptors in the kidney. Our in vitro data are in accordance with findings from other studies, in which irbesartan has been shown to have a higher affinity for the AT₁ receptor than losartan in rat liver membranes and human aortic smooth muscle cells [27,28].

The reasons for this apparent discrepancy between the in vivo and in vitro data for irbesartan are unclear. It is possible that irbesartan may have a lower oral bioavailability as compared with losartan; however, this is highly unlikely, as plasma levels of irbesartan were significantly elevated after oral dosing and tended to be higher and persist longer than those of losartan (or EXP3174). Moreover, this finding is consistent with the known high oral bioavailability of irbesartan of 60–80% [43], as compared with 33% for losartan [24,44].

There were time-dependent differences between irbesartan and losartan which may help to explain the anomaly between the in vivo and in vitro inhibition of AT₁ receptor binding. The apparent reduced potency of irbesartan as compared with losartan may be due to its slower onset of action. For an equivalent 10 mg/kg dose, irbesartan also produced near-complete inhibition of AT₁ receptor binding in the rat kidney, but at between 2 and 8 h, as compared with 1–2 h for losartan. The time course of 2–8 h for maximal inhibition of angiotensin II
receptor binding in the rat kidney in vivo by irbesartan parallels the maximal blood pressure fall in humans for irbesartan, which occurs between 3 and 6 h after oral dosing [22,45]. It is important to note that both irbesartan and losartan produced sustained tissue inhibition of AT₁ receptor binding at 24 h, suggesting a long duration of action. These in vivo results demontrate that blockade of angiotensin receptors in the kidney by irbesartan and losartan is not overcome, and that the time course of receptor blockade is more accurately reflected, which cannot be readily ascertained by in vitro studies.

Irbesartan is approximately 1.5 times more lipophilic than losartan, and 100 times more so than EXP3174 [46]. The slower onset of action of irbesartan would not appear to be due to lower rate of absorption from the gut or first-pass metabolism, but may reflect more specifically the rate of tissue accessibility. Indeed, plasma concentrations of irbesartan were substantially elevated 1–2 h after dosing and remained elevated over a 24-h period. It is clear that the oral bioavailability of irbesartan is at least similar to, if not higher than, that of losartan. It is possible that irbesartan, once absorbed into the systemic circulation, undergoes redistribution or is shunted away to other bodily compartments before gaining access to the kidney and reaching tissue equilibrium. These findings are consistent with the fact that, in humans, irbesartan has a high and rapid oral bioavailability, with plasma concentrations peaking at 1.5–2 h after administration, but the maximal fall in blood pressure occurs 3–6 h after dosing [22,45].

As might be anticipated, both irbesartan and losartan elevated plasma renin activity following oral administration, which was presumably the result of disruption of the AT₁-receptor-mediated inhibition of renin release. However, it is curious that, at 24 h, plasma renin activity and plasma drug levels for losartan fell substantially and tended to return towards normal pre-drug levels. In contrast, plasma drug levels and plasma renin activity were still significantly elevated at 24 h after oral dosing with irbesartan, suggesting a longer half-life and sustained action of this drug. This finding may reflect the known long half-life of irbesartan of 11–15 h, compared with 2 h for losartan (or 6–9 h for EXP3174) [17,22,45]. It is unclear, however, why plasma renin activity fell sharply at 24 h for losartan in particular, despite persistent inhibition of AT₁ receptors in the kidney.

It is important to bear in mind that the potency and efficacy of a drug are mutually exclusive entities. Potency relates to the dose or concentration of a drug required to produce an effect, whereas efficacy is how effectively a drug exerts an effect. Although higher doses of irbesartan may be required to exert its anti-hypertensive effect, studies have shown that, at therapeutically equivalent doses, irbesartan lowers blood pressure more effectively than losartan ([47,48]; see also [49]). At recommended starting (150 mg and 50 mg) or maximum (300 mg and 100 mg) doses for irbesartan and losartan respectively, irbesartan reduced diastolic and/or systolic blood pressure in patients with mild-to-moderate hypertension to a greater extent than did losartan ([47,48]; see also [49]). Similarly, at recommended starting doses, irbesartan (150 mg) reduced the pressor response to exogenous angiotensin II in normotensive humans more effectively than did losartan (50 mg) [50]. The enhanced efficacy of irbesartan in the clinical situation may be due to the partial insurmountable nature of antagonism provided by irbesartan.

In summary, we have shown, following oral administration, the dose–response relationship and time course of angiotensin II receptor inhibition in the kidney by irbesartan, as compared with losartan, by quantitative in vitro autoradiography using the radioligand [125I]-[Sar¹, Ile⁸]angiotensin II. The findings of the present study suggest that irbesartan and losartan produce effective and sustained inhibition of AT₁ receptors in vivo in the kidney following oral administration. However, irbesartan appears less potent, with respect to dosage, than losartan in vivo, despite having a higher affinity for AT₁ receptors in vitro. The reason for this apparent discrepancy is unclear, but it may reflect the slower onset of action of irbesartan and its rate of tissue accessibility. Inhibition of angiotensin II receptors in target tissues, such as the kidney, may represent an important action of AT₁ receptor antagonists which may contribute to the beneficial effects of these agents in the clinical setting.

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