Mechanistic Differences of Various AT₁-Receptor Blockers in Isolated Vessels of Different Origin

Peter Morsing, Gunnel Adler, Ulla Brandt-Eliasson, Linda Karp, Kristina Ohlson, Lars Renberg, Per-Ove Sjöquist, Tommy Abrahamsson

Abstract—The functional inhibitory characteristics of the angiotensin II type 1 receptor blockers (ARB) candesartan; irbesartan; and losartan and its active metabolite EXP 3174 (EXP) were studied in rabbit aortic strips and rat portal vein preparations in vitro. Moreover, plasma-protein binding was determined, and the binding was high (>98.5%) for all ARBs. These values were needed to relate the concentrations of the ARBs used in vitro to the nonprotein bound concentrations in clinical use. In both vascular preparations, candesartan caused a marked decrease in the maximal contractile response of the angiotensin II (Ang II) concentration-response curve. Losartan, EXP, and irbesartan caused a rightward parallel shift without any major effects on the maximal response to Ang II. The inhibitory effect of candesartan developed slowly (maximal effect after >30 minutes) and lasted >2 hours despite repeated washing of the vessels. The effect of losartan, irbesartan, and EXP had a faster onset, and most of the inhibitory effect disappeared after washing. The duration of the inhibitory effects of the ARBs were not related to lipophilicity of the compounds. Cooling of the rat portal vein preparations to 4°C before administration of candesartan prevented the persistent inhibition of Ang II response seen at 37°C. For the other ARBs studied, the magnitude of inhibition and the speed of recovery of the Ang II response were independent of the incubation temperature before washing. In addition, when candesartan was given to conscious rats, the inhibitory effect on Ang II–induced blood pressure responses persisted during the 24-hour period despite nondetectable plasma concentrations of candesartan at 24 hours. It is concluded that functional inhibitory characteristics of candesartan differ from those of the other ARBs tested. At clinically relevant concentrations, candesartan is an insurmountable and long-lasting antagonist of the vascular contractile responses to Ang II. (Hypertension. 1999;33:1406-1413.)

Key Words: receptors, angiotensin aorta portal vein protein binding pharmacology

Selective angiotensin II subtype I receptor blockers (ARB) have been documented as effective and well-tolerated antihypertensive drugs.¹⁻³ The tolerability of these compounds seems to be better than that of other antihypertensives, whereas the blood pressure–lowering effect is about the same as for angiotensin-converting enzyme inhibitors and calcium antagonists.¹⁻³ Regarding efficacy of different ARBs, candesartan and irbesartan were shown to be more effective than losartan in lowering 24-hour blood pressure (BP) in mildly to moderately hypertensive patients.⁴⁻⁵

Candesartan was described to dissociate slowly from AT₁-receptors in cell membrane preparations and to cause a more persistent inhibition of the angiotensin II (Ang II)–mediated vascular contractile response when compared with losartan.⁶ In isolated vascular preparations from the rabbit, losartan caused a parallel rightward shift in the concentration-effect curves for Ang II,⁷ whereas candesartan caused a marked suppression of the maximum contractile response to Ang II.¹,⁶ Thus ARBs may differ in their antagonism of the Ang II–mediated response, causing surmountable or insurmountable antagonism.⁸ The mechanism of the insurmountable antagonism of candesartan and the long-lasting duration of effect is not clear, although it may be related to its slow dissociation from the receptor.¹,⁶

The aim of our study was to investigate the functional inhibitory characteristics of different ARBs. Isolated vascular preparations from the rat and rabbit were used to study the contractile responses to Ang II in the presence of the ARBs candesartan, irbesartan, and losartan and its active metabolite EXP 3174 (EXP). Of special interest was examination of the duration of the inhibition of Ang II–mediated responses, for example, how the blockade persisted after extensive washing of the vascular preparation. A second aim was to compare the antagonistic properties of the ARBs in vascular tissues of different origin, the rabbit aorta, and the rat portal vein, which have different degrees of AT₁-receptor reserves.⁹ In this study, the ARB concentrations selected for the isolated vessel preparations were based on the nonprotein-bound (free) plasma concentrations obtained for each drug in the clinical use of these drugs. Finally, candesartan was given to con-
scious rats to study the relation between inhibition and exogenous Ang II and the drug plasma concentration over a 24-hour period.

Methods

Chemicals
Candesartan, candesartan cilexetil, losartan, EXP (Astra Hässle AB), and irbesartan (Sanofi Rech) were used. PEG/EtOH/H$_2$O; 40/10/50%, was used as vehicle except for the study of protein binding, for which 10% ethanol in phosphate buffer (pH 7.4) was used.

Experimental Procedure
All animal experiments were conducted in accordance with Swedish legislation, and the studies were approved by the Swedish National Board for Laboratory Animals.

Rabbit Aortic Strips
Aortic rings (2 mm in length: 6 to 8 rings/rabbit) from male New Zealand White rabbits (3.0 to 3.4 kg; HB Lidköpings Kaninfarm, Lidköping, Sweden) were prepared and set up for measurements according to previously described methods. In short, the rings were mounted on a force-displacement transducer in a 40-mL organ bath containing a modified Krebs buffer (pH, 7.4), which was maintained at 37°C. The force signal was collected on a recorder and fed into a computerized system. The venous catheter was subsequently connected to a syringe pump.

Duration of Inhibitory Effect, Portal Vein
After calculation of the baseline integrated mean force developed by the smooth muscle vessel preparation, Ang II (3 nmol/L) was added, and the integrated contractile response during the first minute was calculated. Two subsequent recordings after Ang II administration, 30 minutes apart, served as control values. ARB or vehicle was then added to the bath after the last rinse, and the effect of Ang II was tested every 30 minutes during a total experimental time of 210 minutes. The continuation of the experiment was designed according to 3 different protocols.

Experimental Protocol
Concentration-Response Curves and Aortic and Portal Vein Preparations
The vascular preparations were exposed to increasing concentrations of Ang II (starting at 0.3 nmol/L) until the maximal contractile effect was achieved (within 4 to 5 minutes). The ARB was added after the vascular tissue was washed and had recovered to baseline. After a 90-minute incubation time with ARB, a second concentration-response curve for Ang II was constructed. In parallel control experiments, vehicle was given before the second Ang II curve was constructed.

Equilibrium Dialysis (Irbesartan)
With the use of the ultrafiltration method, the protein binding of irbesartan markedly differs from previously reported results. A second technique, equilibrium dialysis, was therefore used to determine the binding. Cells with 2 compartments divided by a Spectra/Por membrane were used. One-milliliter aliquots of spiked plasma, 3 μmol/L, and isotone phosphate buffer (pH 7.4) were added to each of the 2 compartments. The cells were gently shaken at 37°C for periods of 1 hour over 1, 2, 4, 6, 10, and 22 hours, whereafter the concentration of irbesartan was determined and unbound drug fraction was calculated.

Determination of Drug Binding to Plasma Proteins

Statistics
The material was tested by 2-way ANOVA. To test the differences between groups, modified t statistics were used. For multiple comparisons with the same variable, the Bonferroni method was used, which states the significant level of p(N), where N is the number of comparisons to be made. Results are expressed as mean ± SEM.

Duration of Inhibitory Effect: Conscious Rats
The animals received either vehicle or 4.9 μmol/kg candesartan cilexetil by oral gavage. Twenty-three hours after the first dose, an arterial plasma sample was obtained, and the BP response to an intravenous bolus administration of Ang II (0.1 nmol/kg) was recorded, a dose that increased BP ~45 mm Hg in control rats. Drug or vehicle was administered by gavage at 24 hours after the first dose. Plasma samples for determination of plasma concentration of candesartan were drawn at 2, 8, and 24 hours after dose, whereas the BP response to intravenous Ang II was recorded at 2, 4, 8, 16, and 24 hours.

Ultrafiltration
All binding determinations were performed on freshly isolated plasma from 3 male and 3 female healthy volunteers. Ten microliters of a stock solution of candesartan, losartan, EXP, or irbesartan was added per milliliter of plasma to give final concentrations between 4 and 8 μmol/L. Spiked plasma was mixed and incubated for 20 minutes. One-milliliter aliquots were centrifuged in Amicon Centrifree devices for 15 minutes at 37°C. Plasma and ultrafiltrates were analyzed by high-performance liquid chromatography (HPLC) with fluorescence detection, and unbound drug fraction was calculated.

Determination of Lipophilicity
The partitioning in octanol-water was determined by use of 2 different methods, the traditional shake-flask method and a titration method. In the shake-flask method, concentrations of the analytes were determined by an appropriate reversed-phase HPLC system.

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values $\pm$ SE. A value of $P<0.05$ was considered to be statistically significant.

Results

Antagonism of the Contractile Response to Ang II in Rabbit Aortic Rings

The effects of the different ARBs on Ang II–evoked contractile responses in rabbit aortic strips are shown in Figure 1, and values are expressed as percentage of vehicle (absolute value $= 21.5 \pm 1.1$ mN). Increasing concentrations of candesartan resulted in a progressive reduction in the maximal response to Ang II. A half-maximal effect of candesartan on the maximal response was observed at 0.03 nmol/L, whereas at the highest concentration (1 nmol/L) complete inhibition of the Ang II response was observed, showing complete, insurmountable antagonism. Preincubation with irbesartan, losartan, and its active metabolite EXP all resulted in a parallel rightward shift of the concentration-response curve. The maximal response to Ang II was not changed for either irbesartan or losartan, whereas a minor reduction ($-15\%$, $P<0.05$) in the maximal response could be seen with EXP.

Antagonism of the Contractile Response to Ang II in Rat Portal Vein Preparation

The effect of ARBs on Ang II–evoked contractile responses in the rat portal vein is shown in Figure 2, and values are expressed as percentage of vehicle (absolute value $= 3.88 \pm 0.46$ mN). Increasing concentrations of candesartan resulted in a progressive decrease in the maximal response to Ang II, showing an insurmountable antagonism. A half-maximal effect of candesartan on maximal response was observed at 0.1 nmol/L. Preincubation with irbesartan, losartan, and its active metabolite EXP all resulted in a parallel rightward shift of the concentration-response curve, with no suppression of the maximal response, which indicates a surmountable antagonism. In contrast, at higher concentrations of losartan and irbesartan, actual potentiation of the responses was observed, which reached statistical significance for irbesartan (30%, $P<0.01$).

Duration of Antagonism of Portal Vein Contractile Response to Ang II

Protocol 1

The effects of increasing concentrations of ARBs for 1 hour on Ang II–induced responses are shown in Figure 3, and values are expressed as percentage of control (absolute value $= 2.25 \pm 0.06$ mN). The effect of candesartan developed gradually over time. Increasing concentrations produced a dose-dependent increase in blockade, with 1 nmol/L resulting in almost complete blockade of the response at 60 minutes. A slight time-dependent increase was also observed for irbesartan and EXP, whereas a steady-state blocking effect of
Losartan was obtained after only 30 minutes of incubation. Increasing concentrations of these blockers also produced a dose-dependent increase in the blockade (Figure 3). The amount of blockade obtained after 1 hour of incubation with candesartan was persistent despite repeated washing regardless of concentration, whereas the effect obtained after 1 hour of incubation with the other ARBs studied was greatly reduced 30 to 60 minutes after washing.

Protocol 2
Time-dependency of inhibition of Ang II responses in the rat portal vein by the different ARBs is shown in Figure 4, and values are expressed as percentage of control (absolute value = 2.59±0.06 mN). The inhibitory effect of 0.3 nmol/L candesartan increased gradually, reaching an almost complete blockade of the Ang II response at 90 minutes. The effect of irbesartan, losartan, and EXP had a rapid onset within <30 minutes, without any further time-dependent increase in the blockade. Further, the antagonistic effect of the different ARBs, except for candesartan, was greatly reduced within 30 minutes of removing the substance and subsequent washing. The blockade by candesartan remained almost constant for the duration of the experiment.

Protocol 3
Results after 30 minutes of incubation with the ARBs at decreased temperature (4°C), followed by washing with drug-free Krebs buffer up to 180 minutes, are shown in Figure 5, with values expressed as percentage of control (absolute value = 3.39±0.08 mN). The low temperature changed the inhibitory characteristics of candesartan. After a 30-minute incubation with candesartan at 4°C, there was a rapid recovery of the response to Ang II when washed with drug-free buffer before the temperature was increased to 37°C. In contrast, there was no temperature-dependent change in the inhibitory characteristics of the other ARBs studied. Because a dramatic change in inhibitory characteristics was observed for candesartan at 4°C, incubation was also performed at 12°C, which resulted in a level of inhibition that was intermediate to that obtained at 4°C and 37°C (Figure 5).

Conscious Rats: Duration of Inhibitory Effect
The relation between BP responses to exogenous Ang II and the plasma concentrations of candesartan after 4.9 μmol/kg is shown in Figure 6. The plasma concentration of candesartan
decreases after dosing, with no candesartan detected at 24 hours. The inhibitory effect of candesartan was almost complete at 2 hours, and 75% of the Ang II response was still inhibited 24 hours after dosing.

Plasma Protein Binding of AT₁-Receptor Blockers
All 4 compounds were extensively bound to human plasma proteins. The fractions of the bound drugs are given in Table 1. The binding of irbesartan was also studied by equilibrium dialysis over time. Equilibrium was achieved after 22 hours with a free fraction of 0.49±0.06%, that is, 99.5% of irbesartan was bound to plasma proteins.

Lipophilicity of AT₁-Receptor Blockers
The distribution ratio of the ARBs differs between candesartan and EXP on the one hand and irbesartan and losartan on the other (Table 2). The magnitude of the distribution constants (KD) and the magnitude and number of the pKₐ's are similar within the groups. As the distribution ratios (D) depend on both the pKₐ's and the KD's, they will also be in approximately the same level within each group. The results show that candesartan and EXP are more hydrophilic at pH 7.4 than both irbesartan and losartan.

Discussion
The main finding in this series of studies is that the ARBs candesartan, irbesartan, losartan, and EXP differ in their antagonistic properties of Ang II–mediated vascular contractile response. In contrast to the other compounds studied, candesartan caused a marked depression of the maximal response to Ang II in the isolated vascular preparations, demonstrating an insurmountable inhibition of the Ang II–mediated response. Furthermore, candesartan produced a long-lasting antagonism of the vascular response to Ang II, demonstrated by maintained inhibition during washing of the in vitro vascular preparations. The long-lasting effect was further supported by a persistent antagonism of the response to exogenous Ang II in the conscious rat despite the marked decrease in plasma candesartan concentrations. The difference in drug-receptor interaction between candesartan and the other ARBs studied is also illustrated by the observation that the incubation at low temperature eliminated the long duration of inhibition exerted by candesartan.

There is a slight confusion in the nomenclature for receptor-ligand interactions. The terms surmountable/insurmountable are sometimes used in the same way as competi-

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Figure 4. Duration of blockade of the vascular contractile response to Ang II (3 nmol/L) by AT₁-receptor blockers. Portal vein was exposed for increasing time periods (solid lines indicate presence of substance) to one concentration of each AT₁-receptor blocker, before subsequent washing in drug-free Krebs buffer. Candesartan 0.3 nmol/L, losartan 30 nmol/L, irbesartan 3 nmol/L, and EXP 3174 1 nmol/L. Shown are mean values and SE, AT₁-receptor blockers n=8 to 15; vehicle n=80.
tive/noncompetitive, which is not correct. Competitive/non-competitive antagonism are related to experimental conditions in which ligand and antagonist are added at the same time to the receptor preparation, whereas surmountable/insurmountable antagonism describe the interaction after a preincubation step with the antagonist. Thus competitive receptor blockers could well be insurmountable if they depress the maximal response to an agonist after preincuba-

tion. There may be different molecular mechanisms for insurmountable antagonism. The insurmountable behavior of substances like candesartan has been suggested to reflect its slow dissociation from the receptor,1,6 its slow removal from the tissue compartment,15 stimulation of receptor internalization,16 or allosteric modulation of the receptor.17 The vascular tissues used in this study, the rabbit aorta and the rat portal vein, both possess Ang II subtype I receptors as the dominat-
ing Ang II receptor.6,7,9,18 Insurmountable antagonism may be more obvious in tissues that have a low receptor reserve.9

**Figure 5.** Effect of temperature during the drug incubation period on speed of recovery of Ang II responses in rat portal vein. Vessels were incubated with each AT1-receptor blocker (candesartan, 1 nmol/L; losartan, 100 nmol/L; irbesartan, 50 nmol/L and EXP 3174, 10 nmol/L) at 4°C (○) or 37°C (□) for a period of 30 minutes, followed by washing in drug-free Krebs buffer (37°C). Dashed line and open symbols represent vehicle. Incubation was also performed at 12°C (■) with candesartan. Shown are mean values ± SE, n = 7 to 8.

**Figure 6.** Plasma candesartan concentration (○) and blockade of Ang II responses (●) after oral administration of 4.9 µmol/kg candesartan cilexetil to conscious rats. An intravenous bolus injection of Ang II that resulted in an arterial BP increase of 45 mm Hg in nontreated rats was given 2, 4, 8, 16, and 24 hours after oral administration of candesartan cilexetil. Plasma candesartan concentrations were measured at 2, 8, and 24 hours after dose. Shown are mean values ± SE, n = 5.

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**TABLE 1.** Approximated Half-Maximal Plasma Concentration, Measured Protein Binding, and Calculated Free Plasma Concentration for Recommended Clinical Daily Doses of Candesartan (8 and 16 mg), Irbesartan (150 and 300 mg), Losartan (50 mg), and EXP 3174

<table>
<thead>
<tr>
<th>Substance</th>
<th>Plasma Concentration</th>
<th>Plasma Protein Binding, %</th>
<th>Free Plasma Concentration, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candesartan</td>
<td>70–135¹</td>
<td>99.73 ± 0.01 (n = 5)</td>
<td>0.2–0.4</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>1800–3600²</td>
<td>99.53 ± 0.01 (n = 7)</td>
<td>8.5–17</td>
</tr>
<tr>
<td>Losartan</td>
<td>300³</td>
<td>98.50 ± 0.09 (n = 8)</td>
<td>4.5</td>
</tr>
<tr>
<td>EXP 3174</td>
<td>250³</td>
<td>99.78 ± 0.01 (n = 3)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Values for plasma concentrations are obtained from previously published results.

Superscript figures are references. Protein binding values are mean ± SE.
TABLE 2. Calculated Logarithms of Distribution Constants and Distribution Ratios for Octanol-Water for Candesartan, Irbesartan, Losartan, and EXP 3174 as a Determination of Lipophilicity

<table>
<thead>
<tr>
<th>Substance</th>
<th>Log K_o</th>
<th>Log D at pH 7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candesartan</td>
<td>3.8±0.01</td>
<td>−1.7±0.06</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>3.6±0.02</td>
<td>1.0±0.10</td>
</tr>
<tr>
<td>Losartan</td>
<td>3.5±0.01</td>
<td>0.8±0.05</td>
</tr>
<tr>
<td>EXP 3174</td>
<td>4.0±0.02</td>
<td>−1.6±0.08</td>
</tr>
</tbody>
</table>

Values are mean±SE. n=2 to 3.

However, the results in this study demonstrate that the depression in the maximal response to Ang II produced by candesartan in the rabbit aorta was essentially duplicated in the rat portal vein, a tissue with a large receptor reserve to Ang II, although a somewhat higher concentration of candesartan was needed to decrease the maximal Ang II response in the portal vein.

Irbesartan and EXP have been claimed to possess insurmountable antagonism of the Ang II response in isolated vascular preparations. In this study, a partially insurmountable effect was observed for all the concentrations of EXP studied in the rabbit aorta, whereas irbesartan did not show such antagonistic behavior in any of the vessels studied. In contrast, irbesartan significantly increased the maximal response to Ang II in the portal vein. Previous studies with candesartan in the isolated rabbit aorta preparation used a preincubation period of 30 minutes, which in our study was shown to be inadequate. Thus an incubation period of at least 90 minutes is needed to obtain the maximal blockade for at least the lower candesartan concentrations studied.

In this study, the ARBs were incubated in the Krebs buffer at various concentrations and for different time-periods, and the vascular contractile responses to Ang II were recorded during the drug exposure time as well as during the washout period. Candesartan caused long-lasting antagonism of the portal vein contractile response to Ang II, as shown by the maintained inhibition during the washout period. In contrast, corresponding experiments with irbesartan, losartan, and EXP showed a rapid recovery of the responses to Ang II during this period. Importantly, these differences between candesartan and the other ARBs studied were independent of the drug concentration and the drug exposure time. However, Panek et al reported that repeated (5 hours) washing of the rabbit aorta, after (10 minutes) preincubation with 10 nmol/L EXP, did not restore the blunted Ang II response. The reason for the marked discrepancy between this observation and our findings with EXP in the portal vein is at present unknown.

The half-life for the inhibitory effect of candesartan persisted for more than 2 hours in the present study, which is considerably longer than the half-life for dissociation of 66 minutes from the receptor in membrane preparations reported by Ojima et al. The tighter binding of candesartan in the vascular preparation as compared with the membranes could point to a more complex candesartan–AT1-receptor interaction in intact cell systems. In this context, it has been suggested by Panek et al that AT1-blockers could be slowly removed from the tissue compartment, cells, or extracellular matrix. A possible greater tissue “binding” of candesartan compared with the other ARBs studied could not be explained by their hydrophobic/hydrophilic characteristics. Indeed, our measurements show that candesartan and EXP are more hydrophilic than losartan and irbesartan.

A conformational change or internalization of the receptor may constitute an alternative explanation for the long-lasting candesartan–receptor coupling in intact cells. This is supported by the finding that the persistent inhibition by candesartan after washout was abolished when this compound was incubated at reduced temperature. In contrast, the other ARBs show a similar rate of recovery at washout after incubation at 4°C and 37°C. These results indicate that the tight binding of candesartan in intact cells represents a dynamic phenomenon requiring either the membrane lipids to be sufficiently fluid, such as for a conformational change in the receptor, or an energy-dependent mechanism such as the internalization of the candesartan-associated receptor.

In this study, the concentration ranges of the ARBs investigated were 0.003 to 10 nmol/L (candesartan); 1 to 100 nmol/L (irbesartan); 1 to 100 nmol/L (losartan); and 0.01 to 10 nmol/L (EXP). To relate these concentration ranges, used in vitro, to the unbound plasma concentrations observed in clinical use of these ARBs, we found it important to measure the extent of plasma protein binding of the compounds. Previous studies have reported very high binding (>98%) of candesartan, losartan, and EXP, whereas irbesartan has been reported to have a plasma protein binding of ~90%. The results from our measurements confirm the extensive plasma protein binding of candesartan, losartan, and EXP. In contrast, using 2 different methods, we observed a significantly greater degree of plasma protein binding for irbesartan (99.5%) than the 90% previously reported. The reason for this discrepancy is unknown, but additional experiments, including an equilibrium dialysis at a second laboratory, confirmed our initial result of a plasma protein binding of ~99.5% for irbesartan (Covance).

The concentrations of drugs for the in vitro experiments were calculated from previously reported drug peak plasma concentrations (Cmax) obtained at the recommended clinical doses of candesartan (8, 16 mg), irbesartan (150, 300 mg), and losartan (50 mg) in humans. The plasma concentration at half Cmax was used together with the measured plasma protein binding values to calculate the free plasma concentrations of the ARBs studied (see Table 1). For candesartan, the free plasma concentrations (at half Cmax) were ~0.3 nmol/L. This concentration markedly depressed the maximal response to Ang II, both in the isolated rabbit aorta and rat portal vein preparations. For losartan, the free plasma concentrations (half Cmax) were ~5 nmol/L (losartan) and ~0.6 nmol/L (EXP). In the isolated vascular preparations, this concentration of losartan only marginally affected the Ang II responses, whereas a much greater effect was seen for EXP at a concentration of 1 nmol/L. Moreover, EXP caused a slight reduction in the maximal response to Ang II in the rabbit aorta, whereas the corresponding rat portal vein experiment showed a maintained maximal response. Altogether, these results suggest that EXP exerts the major AT1-receptor blocking effect in humans after administration of 50 mg.
losartan and that losartan may be regarded as a prodrug metabolized to a more active ARB. For irbesartan, the free plasma concentration (half C\text{\textsubscript{max}}) was \(\sim 10\) nmol/L, a concentration that caused a marked rightward shift of the concentration-response curve to Ang II without affecting the maximal response.

The long duration of inhibition obtained with candesartan also could be demonstrated in the study in conscious rats. The results show clearly that the inhibitory effect of candesartan on exogenous Ang II administration lasts well after the plasma concentration of the drug has reached nondenotactive levels. The C\text{\textsubscript{max}} of candesartan in rats reached with this dose (4.9 \(\mu\)mol/kg) is compatible with what would be expected at C\text{\textsubscript{max}} after a dose of 16 mg to humans. Moreover, the finding of persistent blockade of the Ang II response, despite decreasing plasma concentrations, is also reported in recent clinical pharmacology studies with candesartan. This series of studies clearly shows that candesartan, at clinically relevant concentrations, behaves like an insurmountable antagonist at the AT\textsubscript{1}-receptor and produces a long-lasting blockade (in vitro and in vivo) of the vascular contractile effects of Ang II. At corresponding concentrations, irbesartan and EXP behave like surmountable antagonists, with a relatively short duration of action in vitro after washout. An important question is whether these differences in antagonistic properties will result in significant differences in the blood pressure–lowering efficacy and/or organ-protective effects. So far this question has only been addressed to a limited extent. Candesartan (16 mg dose) was more effective than losartan (50 mg dose) in lowering (24 hours) BP in mildly to moderately hypertensive patients and in healthy volunteers during salt restriction. There are no studies so far comparing irbesartan and candesartan, whereas a recent study has shown irbesartan (300 mg) to be superior to losartan (100 mg) in antihypertensive effects.

It is concluded that the ARBs studied differ in their antagonistic properties of Ang II–mediated contractile effects in the isolated rabbit aorta and the rat portal vein. At clinically relevant concentrations, candesartan, in contrast to the other ARBs studied, caused a marked depression of the maximal response to Ang II and long-lasting antagonism of the vascular contractile response to Ang II.

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