Effects of acute AT$_1$ receptor blockade by candesartan on arterial pressure and renal function in rats

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Cervenka, Ludek, Chi-Tarng Wang, and L. Gabriel Navar. Effects of acute AT$_1$ receptor blockade by candesartan on arterial pressure and renal function in rats. Am. J. Physiol. 274 (Renal Physiol. 43): F940–F945, 1998.—Experiments were performed on normal anesthetized rats to determine the effects of candesartan, a novel AT$_1$ receptor antagonist, on the arterial pressure and renal hemodynamic responses to bolus doses of angiotensin II (ANG II) and on renal hemodynamics and sodium excretion. Control arterial pressure responses to bolus ANG II doses of 10, 50, 100 and 1,000 ng were 26 ± 6, 54 ± 7, 57 ± 7, and 79 ± 7 mmHg; the decreases in cortical renal blood flow (CRBF), measured with laser-Doppler flowmetry, were 47 ± 9, 64 ± 8, 71 ± 6, and 82 ± 6%. The vasoconstrictor responses to ANG II up to 1,000 ng were completely blocked by candesartan doses of 1 and 0.1 mg/kg, whereas treatment with 0.01 mg/kg candesartan attenuated the arterial pressure and CRBF responses. The higher doses of candesartan (1 and 0.1 mg/kg) elicited rapid decreases in arterial pressure, leading to associated decreases in sodium excretion. Renal blood flow (RBF), glomerular filtration rate (GFR), and urine flow also decreased following treatment with candesartan at 1 mg/kg. In contrast, when candesartan was given at 0.01 mg/kg, which did not decrease arterial pressure significantly, there were significant increases in GFR (16 ± 4), RBF (9 ± 2), urine flow (11 ± 2), sodium excretion (35 ± 7), and fractional sodium excretion (39 ± 8%). The inability to overcome blockade, even with very high ANG II doses, indicates that candesartan is a potent noncompetitive blocker of ANG II pressor and renal vasoconstrictor effects. The lower candesartan dose that did not cause significant hypotension elicited substantial increases in RBF, GFR, and sodium excretion, revealing the direct renal vasodilator and natriuretic effects of AT$_1$ receptor blockade.

AT$_1$ receptor blockade; glomerular filtration rate; renal blood flow; sodium excretion

THE DEVELOPMENT OF nonpeptide angiotensin II (ANG II) receptor antagonists has allowed more detailed evaluation of the role of ANG II in the regulation of renal function, arterial blood pressure, and sodium excretion under normal and hypertensive conditions (2, 5, 14, 26). However, previous results from studies using various pharmacological inhibitors or antagonists of the renin-angiotensin system (RAS) to assess the prevailing influence of endogenous ANG II on renal function and sodium excretion have not yielded a consistent pattern (18). Although renal blood flow (RBF) is generally increased, the glomerular filtration rate (GFR) responses to inhibition of the RAS have been much more variable. Indeed, GFR has been reported to be increased (3, 25, 32), unchanged (2, 8), or decreased (9, 16) following pharmacological inhibition of the RAS. However, systemic ANG II blockade often causes substantial decreases in arterial pressure, and the GFR responses to inhibition of the RAS may be, in large part, dependent on the associated decreases in arterial blood pressure. Thus specific hemodynamic and sodium excretory responses in normotensive rats to AT$_1$ receptor blockade have remained uncertain because of the confounding effects of associated changes in arterial pressure and the possible activation of compensatory mechanisms such as the sympathetic nervous system (7, 24).

Recent studies have indicated that the novel AT$_1$ receptor antagonist, CV-11974 (candesartan), is a highly potent AT$_1$ receptor antagonist without agonistic properties (22). Candesartan reduced arterial pressure in a dose-related manner in various hypertensive models, such as spontaneously hypertensive rats (SHR); two-kidney, one-clip (2K1C) Goldblatt hypertensive rats; and one-kidney, one-clip hypertensive rats (12). It has been suggested that candesartan causes vasodilatation of the renal vasculature in conscious SHR and in 2K1C Goldblatt hypertensive rats (29, 30). However, detailed renal functional responses to candesartan have not been reported. Thus, in the present study, we determined the effects of depressor and nondepressor doses of candesartan on renal function and urinary sodium excretion in normotensive rats. Specific attention was focused on the renal functional and excretory responses to candesartan at a dose that did not cause substantial reductions in systemic arterial pressure. To assess the degree of blockade, the effects of candesartan treatment at these doses (0.01–1 mg/kg iv) on the arterial blood pressure and cortical renal blood flow (CRBF) responses to bolus doses of ANG II were characterized.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River, Wilmington, MA) were housed in a temperature- and light-controlled room and allowed access to standard rat chow (Ralston Purina, St. Louis, MO) and water ad libitum. On the day of experiment, rats weighing 265–320 g were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed on a thermoregulated table so that body temperature could be maintained at 37–37.5°C. A tracheostomy was performed to maintain a patent airway, and the exterior end of the tracheal cannula was placed inside a small plastic chamber into which humidified 95% O$_2$–5% CO$_2$ was continuously passed. The right jugular vein was catheterized with PE-50 and PE-10 catheters for infusion of solutions and pentobarbital sodium as needed to maintain an appropriate level of anesthesia. The right femoral artery was cannulated and connected to a Grass Polygraph (Grass Instrument, Quincy, MA) via a Statham pressure transducer for arterial pressure monitoring. The right femoral vein was cannulated to provide a separate access for intravenous bolus doses of ANG II.

The left kidney was exposed via a flank incision, isolated from surrounding tissue, and placed in a Lucite cup to keep it stable. The tip of a laser-Doppler flow probe (Med Pacific, Seattle, WA) was placed close to the surface of the kidney for measurement of relative changes in CRBF. The laser-Doppler
flow technology allows dynamic assessment of relative changes in RBF (4, 28). The ureter was cannulated with a PE-10 catheter. During the surgical and preparative procedures, an isotonic saline solution containing albumin (6 g/dl) was infused at a rate of 20 µl/min. After surgery, an isotonic saline solution containing albumin (1 g/dl), p-aminohippurate sodium (PAH; Merck Sharpe & Dohme, West Point, PA) (1.5 g/dl), and inulin (Inutest; Laevosan, Linz, Austria) (2 g/dl) was infused at the same infusion rate. After completion of surgery, a 1-h equilibration period was allowed.

The experimental protocol consisted of two 30-min clearance periods to assess control renal function. The rats then received a single intravenous injection of candesartan. After a 10-min delay, two 30-min experimental clearance periods were performed. Two blood samples were collected at the midpoints to calculate inulin and PAH clearances and to assess the renal functional responses to candesartan treatment. The responses in arterial pressure and CRBF to bolus doses of ANG II (10, 50, and 100 ng) were tested at the beginning of the experiment; after candesartan treatment, the blood pressure and CRBF responsiveness to bolus doses of ANG II (10, 50, 100, and 1,000 ng) were tested again. At the end of each experiment, the left kidney was removed, blotted dry, and weighed to normalize the data per gram of kidney weight. The following experimental groups were examined: group 1, 1 mg/kg iv candesartan (n = 5); group 2, 0.1 mg/kg iv candesartan (n = 5); group 3, 0.01 mg/kg iv candesartan (n = 4); and group 4, vehicle control with intravenous saline (n = 4). All drugs were given in total volume 100 µl.

We performed additional experiments to address the potential role of AT$_2$ receptors in the modest vasodilatory response to ANG II after treatment with the highest dose of candesartan. Rats were pretreated with candesartan (1 mg/kg). After 15 min, we tested the responses to bolus doses of ANG II (50, 100, and 1,000 ng). Then we gave a dose of the AT$_2$ receptor antagonist (PD-123319, 5 mg/kg), and, 15 min later, we again tested the responses to bolus doses of ANG II (50, 100, and 1,000 ng).

Analytic procedures. Blood and urine samples were analyzed for inulin, PAH, sodium, and potassium concentrations. Inulin and PAH concentrations were measured colorimetrically. Sodium and potassium concentrations were determined by flame photometry. Hematocrits were assessed on each blood sample.

Calculations and statistical analyses. GFR was calculated from urine and plasma inulin concentrations and urine flow. PAH clearance was used as an index of renal plasma flow (RPF). RBF was estimated from the PAH clearance and hematocrit values but without correction for PAH extraction. Urine sodium concentration and urine flow were used to calculate the sodium excretion rate, and fractional sodium excretion was calculated from the ratio of urine and plasma sodium concentrations divided by the urine-to-plasma inulin ratio. Fractional potassium excretion was calculated from the ratio of urine and plasma potassium concentrations divided by urine and plasma inulin ratios. Results are expressed as means ± SE. Statistical comparisons within groups were conducted using analysis of variance for repeated measures (ANOVA), followed by Newman-Keuls test. Unpaired t-test was used for comparisons between groups. Values exceeding the 95% critical limits (P < 0.05) are considered to be statistically significant.

**RESULTS**

Effects of acute candesartan on arterial pressure. As shown in Fig. 1, candesartan doses of 1 and 0.1 mg/kg iv led to progressive decreases in mean arterial pressure (MAP) over the course of 70 min (123 ± 2 to 96 ± 4 and 122 ± 3 to 100 ± 8 mmHg; P < 0.05, respectively). Administration of candesartan at a dose of 0.01 mg/kg did not reduce arterial pressure significantly in comparison with rats treated with the saline vehicle alone (117 ± 7 to 114 ± 9 and 112 ± 5 to 111 ± 5 mmHg, respectively).

Effects of candesartan on arterial pressure responses to bolus doses of ANG II. As shown in Fig. 2, the control responses in arterial pressure to bolus doses of ANG II at doses of 10, 50, 100, and 1,000 ng were 26 ± 6, 54 ± 7, 57 ± 7, and 79 ± 7 mmHg. Candesartan treatment at 1–0.1 mg/kg completely blocked the pressor responses to ANG II up to 1,000 ng, although there were slight, but not significant, increases in arterial pressure (6 ± 2 mmHg) in the rats treated with 0.1 mg/kg candesartan. After treatment with the highest candesartan dose, the ANG II bolus dose (1,000 ng) actually decreased arterial pressure (−5 ± 2 mmHg), suggesting a slight vasodilatory effect. This vasodilatory effect of ANG II was prevented by treatment with the AT$_2$ receptor blocker (PD-123319). Candesartan treatment at 0.01 mg/kg markedly attenuated but did not completely block the arterial pressure responses to bolus doses of ANG II to 9 ± 3, 12 ± 3, 17 ± 5, and 23 ± 6 mmHg.

Effects of candesartan on CRBF responses to bolus of ANG II. As shown in Fig. 3, the control decreases in CRBF to ANG II doses of 10, 50, 100, and 1,000 ng averaged 47 ± 9, 68 ± 8, 81 ± 6, and 82 ± 6%, respectively. The CRBF responses were completely blocked by candesartan at doses of 1 and 0.1 mg/kg. Candesartan at a dose of 0.01 mg/kg markedly attenuated but did not abolish the decreases in CRBF to ANG
Effects of candesartan on RBF. As shown in Fig. 4, top, the highest candesartan dose of 1 mg/kg led to significant decreases in RBF from $5.1 \pm 0.7$ to $2.3 \pm 0.6$ ml·min$^{-1}$·g$^{-1}$ ($P < 0.05$). These decreases were closely associated with the decreases in systemic arterial pressure, and the CRBF data showed a decrease of $23 \pm 7\%$ ($P < 0.05$).
3% within the first 15 min. Candesartan at a dose of 0.1 mg/kg elicited slight decreases in RBF from 5.0 ± 0.2 to 4.3 ± 0.7 ml·min⁻¹·g⁻¹, but this decrease was not statistically significant. Likewise, CRBF was not significantly altered at this dose. In contrast, the lowest dose of candesartan (0.01 mg/kg) led to significant increases in RBF from 5.5 ± 0.4 to 6.0 ± 0.1 ml·min⁻¹·g⁻¹ (P < 0.05). This was also followed by changes in outer CRBF, which increased by 25 ± 9%. Time-control rats (given only the vehicle) did not show a change in RBF (6.2 ± 1.0 to 6.3 ± 1.0 ml·min⁻¹·g⁻¹).

Effects of candesartan on GFR. As shown in Fig. 4, bottom, GFR decreased significantly in the rats treated with the highest dose (1 mg/kg) from 0.9 ± 0.2 to 0.4 ± 0.1 ml·min⁻¹·g⁻¹ (P < 0.05). However, the GFR did not change significantly following treatment with an intermediate dose of 0.1 mg/kg nor in the rats given the saline vehicle. As with the RBF responses, candesartan at a dose of 0.01 mg/kg elicited significant increases in GFR from 1.0 ± 0.1 to 1.2 ± 0.1 ml·min⁻¹·g⁻¹ (P < 0.05).

Effects of candesartan on urine flow and on sodium excretory function. The rats treated with the 1 mg/kg dose of candesartan exhibited significant decreases in urine flow from 5.9 ± 0.6 to 4.3 ± 0.3 µl/min (P < 0.05). However, no significant changes in urine flow occurred in the rats treated with the 0.1 mg/kg dose or in the rats serving as time and vehicle controls (6.6 ± 0.2 to 6.5 ± 0.2 and 6.6 ± 0.3 to 6.7 ± 0.2 µl/min, respectively). As with GFR and RBF responses, urine flow in the rats treated with the lowest dose of candesartan increased significantly from 6.4 ± 0.1 to 7.1 ± 0.1 µl/min (P < 0.05).

As shown in Fig. 5, the candesartan doses of 1 and 0.1 mg/kg caused significant decreases in absolute sodium excretion [0.17 ± 0.02 to 0.08 ± 0.01 and 0.21 ± 0.1 to 0.11 ± 0.01, µeq/min (P < 0.05), respectively] and in fractional sodium excretion [0.19 ± 0.03 to 0.09 ± 0.01 and 0.19 ± 0.02 to 0.12 ± 0.01%, respectively (P < 0.05)]. In contrast, the candesartan dose of 0.01 mg/kg elicited significant increases in sodium excretion [0.17 ± 0.01 to 0.23 ± 0.02 µeq/min (P < 0.05)] and in fractional sodium excretion [0.18 ± 0.02 to 0.26 ± 0.03% (P < 0.05)]. In the time control group, sodium excretion and fractional sodium excretion did not change. No significant changes in fractional potassium excretion were found in any of the groups of rats before or after candesartan treatment.

**DISCUSSION**

Although there have been numerous studies evaluating the effects of ANG II receptor antagonists on renal function, the results have been quite variable, with both decreases and increases in RBF, GFR, and sodium excretion being reported (2, 3, 15, 16, 19, 25, 32). The decreases in renal function have often been explained by the associated decreases in MAP, along with the accompanying increases in renal sympathetic nerve activity (20, 24), but there has not been a detailed delineation of the renal responses to AT₁ receptor blockade in the absence of substantial decreases in arterial pressure. The more recent availability of the highly potent AT₁ receptor antagonists with reportedly noncompetitive characteristics prompted evaluation of AT₁ receptor blockade on renal function with emphasis on a comparison of renal responses under conditions where arterial pressure was maintained vs. responses when hypotension resulted.
Our initial studies with candesartan confirmed its highly potent actions on arterial pressure and renal function. These data demonstrate that the arterial blood pressure responses to ANG II were completely blocked with the highest dose of candesartan. Even with the lowest dose of candesartan, which did not reduce arterial pressure significantly, ANG II at 1,000 ng bolus failed to overcome the blockade on arterial pressure and on the renal vasculature. These results are consistent with the previous suggestions that candesartan functions as a noncompetitive AT₁ receptor antagonist (22).

Of interest was the observation that the highest candesartan dose (1 mg/kg) not only blocked the vasopressor response to the highest dose of ANG II but also resulted in a modest vasodepressor response, in that the arterial blood pressure was decreased significantly by the ANG II (−5 ± 2 mmHg). These observations suggest that candesartan does not block AT₂ receptors, which presumably mediated the slight vasodilatation observed in response to ANG II during complete AT₁ receptor blockade. It has been shown that ANG II bolus doses have biphasic effects on arterial blood pressure. They initially elicit pressor effects followed by depressor actions. The initial pressure responses have been blocked with AT₁ receptor antagonists, whereas the depressor effects have been blocked by AT₂ receptor antagonists (21). Also, it has been demonstrated that the targeted disruption of the mouse AT₂ gene resulted in an increased sensitivity to the pressor action of ANG II (10, 11). Therefore, it seems likely that, in the present study, the specific, noncompetitive AT₁ receptor antagonist, candesartan, unmasked an AT₂ receptor-mediated vasodilatation in response to ANG II bolus. This was supported by the experiments in rats treated with AT₂ receptor antagonist (PD-123319). In these rats, the highest dose of ANG II failed to elicit any vasodilatation effects after treatment with candesartan. It should also be noted that, since the highest dose of ANG II did not elicit renal vasodilatation in the rats treated with the highest dose of candesartan, a role for AT₁ receptors in the modulation of renal hemodynamics was not apparent. In our study, candesartan caused an immediate decrease in mean arterial pressure (MAP) within 5 min; however, the maximum depressor effect was achieved 50 min later. This is in agreement with observations of Xiao and Widdop (30), which demonstrated the immediate action of candesartan on MAP after 3 min in SHR and Wistar-Kyoto (WKY) rats. In their study, candesartan caused only a small reduction in MAP in WKY rats. However, their study was performed on conscious animals, and our study was performed on anesthetized animals. Because the effects of pentobarbital sodium anesthesia on activation of RAS are well known (27), it is conceivable that the effects of AT₁ blockade on MAP are magnified.

During our initial studies employing the higher doses of candesartan, we observed decreases in RBF, GFR, sodium excretion, and fractional sodium excretion. As reported before (2), these responses were associated with substantial decreases in MAP. The decrease in MAP leading to hypotension probably decreased RBF, GFR, and sodium excretion directly, as well as indirectly, as a consequence of increased renal sympathetic nerve activity (7, 24, 31). Indeed, the importance of the RAS has been shown in studies where ANG II blockade was not associated with major reductions in MAP. Significant increases in renal plasma flow, GFR, sodium excretion, and urinary flow were observed when a combination of renin inhibitor, an angiotensin-converting enzyme inhibitor and an ANG II receptor antagonist were infused directly into the renal artery of dogs. However, these changes did not occur when this combination of inhibitors was infused systemically and was followed with a decrease in systemic pressure (23). Thus decreases in renal function following systemic AT₁ are probably indirectly mediated as a consequence of activation of compensatory mechanisms, including stimulation the sympathetic nervous system to minimize the decreases in arterial pressure (7, 24, 31).

The lowest dose of candesartan (0.01 mg/kg), which did not decrease arterial pressure significantly, still exhibited effective AT₁ receptor blockade and was sufficient to elicit significant increases in GFR, RBF, urine flow, sodium excretion, and fractional sodium excretion. The increases in fractional sodium excretion, as well as total sodium excretion, suggest that, in addition to the natriuresis caused by the renal hemodynamic changes, blockade of tubular AT₁ receptors contributed to the increases in urinary sodium excretion (6, 17). The increases in GFR could have been due to both the vasodilatory actions on the renal microvasculature, as well as to increases in the glomerular filtration coefficient due to blockade of the effect of endogenous ANG II at the glomerulus (1).

In summary, the results indicate that candesartan is a potent, specific, and noncompetitive blocker of ANG II pressor and renal vasoconstrictor effects. ANG II receptor blockade, under conditions where the decrease in MAP is minimal, elicits substantial increases in RBF and GFR and proportionally much greater increases in sodium excretion. These responses reveal the direct renal effects of AT₁ blockade on renal hemodynamic and excretory responses.

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