Prevention of Hypertension by Irbesartan in Dahl S Rats Relates to Central Angiotensin II Type 1 Receptor Blockade

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Abstract—Hypertension in Dahl S rats on high-salt intake is in general considered a model of “low-renin hypertension,” unresponsive to treatment with blockers of the renin-angiotensin system. However, direct central administration of an angiotensin II type 1 (AT₁) receptor blocker prevents both the sympathoexcitation and hypertension caused by high-salt intake in Dahl S rats. In the present study, we tested the hypothesis that chronic peripheral administration of an AT₁ receptor blocker inhibits the salt-induced hypertension relative to the extent of central AT₁ receptor blockade that is induced. Dahl S rats received a high-salt (1370 μmol Na⁺/g) or regular (101 μmol Na⁺/g) diet from 4 to 8 weeks of age. In 3 different sets of experiments, Dahl S on high salt were randomized to intracerebroventricular (ICV) treatment with control infusion versus irbesartan at 50 or 250 μg · kg⁻¹ · d⁻¹, oral treatment with control versus irbesartan at 125 or 500 mg · kg⁻¹ · d⁻¹ once daily by gavage, or subcutaneous treatment with control versus irbesartan at 50 or 150 mg · kg⁻¹ · d⁻¹ by once daily injection. At 8 weeks of age, MAP was measured in conscious rats at rest and in response to angiotensin II ICV or IV. On high-salt intake, Dahl S developed the anticipated marked increase in MAP to ≈160 mm Hg. Irbesartan ICV did not affect pressor responses to angiotensin II IV, but irbesartan administered subcutaneously or by gavage markedly inhibited these responses. Irbesartan ICV or by gavage partially inhibited pressor responses to angiotensin II ICV and the development of hypertension. Irbesartan subcutaneously at the higher dose more completely inhibited pressor responses to angiotensin II ICV and fully prevented the salt-induced hypertension. The degree of central but not peripheral AT₁ receptor blockade parallels the antihypertensive effect of irbesartan, indicating that inhibition of the brain renin-angiotensin system can contribute to a significant extent to the therapeutic effectiveness of AT₁ receptor blockers such as irbesartan when administered in sufficiently high doses to cause central AT₁ receptor blockade. (Hypertension. 2001;37:981-984.)

Key Words: Rats, Dahl ■ hypertension, sodium-dependent ■ brain ■ renin-angiotensin system ■ receptors, angiotensin II ■ irbesartan

Recent studies have demonstrated that the brain renin-angiotensin system (RAS) plays a pivotal role in the sympathoexcitation and hypertension caused by high-salt intake in animal models of salt-sensitive hypertension. Although it is not yet known whether (and where) angiotensin (Ang) II or III increases, Ang II type 1 (AT₁) receptor stimulation appears to be essential, because chronic intracerebroventricular (ICV) infusion of AT₁ receptor blockers prevents the sympathoexcitation and hypertension by high-salt intake in both spontaneously hypertensive rats (SHR) and Dahl salt-sensitive (Dahl S) rats.¹⁻³ In contrast, the circulatory RAS is suppressed during the development of hypertension in Dahl S on high-salt intake,⁴⁻⁶ and this model of “low-renin hypertension” is in general considered to be unresponsive to treatment with blockers of the RAS. Most studies with chronic peripheral (subcutaneous or oral) treatment with ACE inhibitors or AT₁ receptor blockers did not show effects on the hypertension induced by high-salt intake.⁷⁻⁹ Some studies did observe some blunting, mainly with higher doses.⁵,¹⁰,¹¹ It is possible in these latter studies that sufficient amounts of the blockers reached the brain, causing blockade of the brain RAS and thereby blunting/prevention of the hypertension. To test this concept, we used chronic treatment with the lipophilic AT₁ receptor blocker irbesartan,¹² by either ICV infusion, once-daily gavage, or once-daily subcutaneous (SC) injection, and related the degree of central versus peripheral blockade (with ICV versus IV Ang II) to the antihypertensive effect in Dahl S rats on high-salt intake.

Methods

Animals and Treatment

Male Dahl S rats (3 to 4 weeks of age) were obtained from Harlan Sprague-Dawley Inc. The rats were housed in a climate-controlled room on a 12-hour light/dark cycle and fed regular rat chow (101 μmol Na⁺/g food) and tap water ad libitum for 5 days before a high-salt diet and irbesartan treatment were started. The animals were allocated to the following study protocols, and within each protocol, they were randomized to specific treatments (6...
to 11 rats per treatment). Doses of irbesartan were selected on the basis of previous studies.\textsuperscript{13–15}

**Protocol 1: Irbesartan by Chronic ICV Infusion**

Under halothane inhalation anesthesia, a 23-gauge guide needle was fixed on the skull over the right lateral ventricle, and a 23-gauge stainless steel right-angled cannula was implanted onto the left lateral ventricle, as previously described.\textsuperscript{16} Infusion of irbesartan at 50 or 250 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) or vehicle (0.84% \( \text{NaHCO}_3 \)) was initiated with osmotic minipumps (12 \( \mu \text{L} / \text{d}, \text{model 2002; Alza}), which were implanted SC and connected to the ICV cannula. One group of rats stayed on a regular sodium diet and received vehicle solution ICV. The other groups were placed on a high-salt (1370 12 \( \mu \text{mol Na} / \text{g food} \)) diet at this time.

**Protocol 2: Irbesartan by Gavage**

Irbesartan was delivered through a gavage tube at 125 or 500 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) once daily in the afternoon to rats on the high-salt diet. Vehicle solution (2 mL/kg 0.84% \( \text{NaHCO}_3 \)) was administered to control rats.

**Protocol 3: Irbesartan by SC Injection**

Irbesartan (50 or 150 mg \( \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) in 1.5% arginine as vehicle) was injected SC once daily in the afternoon. Control rats received vehicle solution by SC injection.

The treatments lasted for 4 weeks. At end of the third treatment week, in the gavage and SC treatment groups an ICV cannula was placed in the right lateral ventricle. In the ICV group, the cannula was implanted with the other ICV cannula (used for chronic ICV infusion) at the beginning of treatment. All experimental procedures were approved and carried out in accordance with the guidelines of the University of Ottawa Animal Care Committee for care and use of laboratory animals.

**Blood Pressure Response to Ang II**

After 4 weeks of treatment, 1 PE-50 catheter was placed into the left carotid artery, and 1 was placed into the right jugular vein. The next morning (=16 hours after the last gavage or SC dosing), resting arterial blood pressure was recorded on an MP100WSW system (Biopac Systems, Inc), and heart rate (HR) was calculated from the blood pressure curves. After measurement of resting BP, responses of BP and HR to Ang II (Sigma Chemical Co) were assessed. Ang II was injected through the ICV catheter at 20 and 40 ng/rat (in 2 \( \mu \text{L} \) 0.9% saline, at a 30-minute interval). Twenty to 30 minutes later, Ang II was injected IV at 30 and 100 ng/animal with a 30-minute interval). Twenty to 30 minutes later, Ang II was injected IV at 30 and 100 ng/animal with a 30-minute interval between the 2 doses. At the conclusion of hemodynamic measurements, rats were deeply anesthetized with sodium pentobarbital and injected ICV with 2 \( \mu \text{L} \) of 25% India ink in saline to verify placements of the ICV cannulas.

**Statistical Analysis**

All values are expressed as mean\( \pm \)SEM. One-way ANOVA was used to determine the effects of treatments and diet on various parameters (SAS Institute). When the \( F \) value was significant, a Duncan’s multirange post hoc test was used to locate the differences between groups. Statistical significance was defined as \( P<0.05 \).

**Results**

**Pressor Responses to Ang II IV**

Ang II IV similarly increased BP in rats on a regular or high-salt diet (Figure 1). Irbesartan ICV did not affect the BP responses to Ang II IV. Irbesartan by gavage markedly reduced the increase in BP, with a slightly greater attenuation at the higher dose. The SC injection of irbesartan at the lower dose significantly attenuated the increase of BP, whereas the higher dose of irbesartan completely blocked the BP responses to the 2 doses of Ang II used.

**Pressor Responses to Ang II ICV**

Ang II ICV increased BP in a dose-related manner (Figures 2, 3, and 4). In Dahl S rats, high-salt intake did not affect these responses. The 2 doses of irbesartan ICV attenuated the pressor response to Ang II ICV by \( \approx 50\% \). The 2 doses of irbesartan by gavage similarly inhibited the pressor response to Ang II ICV by 30% to 50%. Subcutaneous irbesartan attenuated the increase of BP by \( \approx 50\% \) at the lower dose and more marked at the higher dose. Ang II ICV caused minor (NS) decreases in HR, by 20 to 30 bpm (data not shown).

**Resting BP and HR**

High-salt intake for 4 weeks caused a significant increase in resting BP, by 30 to 40 mm Hg, in all 3 study protocols (Figures 2, 3, and 4). Irbesartan ICV caused a 50% attenuation.
tion of the increase in BP with high salt. Irbesartan by gavage similarly attenuated the increase in BP with high-salt intake, which was not significantly different for the low and higher doses. The lower dose of irbesartan SC significantly attenuated the increase in BP, whereas the higher dose of irbesartan SC fully prevented the increase in BP by high salt. HR was similar in all groups (data not shown).

**Discussion**

The present study provides a major new finding that the development of hypertension in Dahl S rats on high-salt intake can be fully prevented by chronic SC treatment with the AT1 receptor blocker irbesartan, presumably because sufficient drug enters the central nervous system (CNS) to inhibit the brain RAS.

**Pressor Responses to ICV Versus IV Ang II**

As anticipated, 16 to 18 hours after dosing, irbesartan by gavage or SC still caused a marked inhibition of the pressor responses to IV Ang II. The lower dose of irbesartan SC (50 mg · kg⁻¹ · d⁻¹) was only partially effective at this point. In contrast, ICV infusion of irbesartan at 50 or 250 µg · kg⁻¹ · d⁻¹ did not cause any detectable inhibition of the pressor responses to IV Ang II. Even if some irbesartan diffused out of the CNS into the periphery, relative to the plasma levels achieved by oral or SC administration at 1000-fold higher doses, plasma levels achieved with ICV dosing would be so low that no relevant inhibition would be expected.

The ICV administration of Ang II caused dose-related increases in BP, likely via activation of neurons in the median preoptic nucleus and juxtaglomerular cells. The former organ and organum vasculosum laminae terminalis. The ICV infusion of irbesartan at 50 or 250 µg · kg⁻¹ · d⁻¹ caused only a 50% inhibition of these pressor responses. In previous studies with losartan at 1 mg · kg⁻¹ · d⁻¹ ICV, more complete blockade of pressor responses to ICV Ang II was found. However, the limited aqueous solubility of irbesartan prevented higher infusion rates to be administered ICV via the osmotic minipump approach.

At the doses used, irbesartan by gavage also caused ≥50% inhibition of the pressor responses to ICV Ang II. In contrast, irbesartan SC once daily at the higher dose caused a nearly complete inhibition of the pressor responses to ICV Ang II at 16 to 18 hours after dosing. Thus, at a 3-fold lower dose, SC irbesartan more effectively induced central AT1 receptor blockade compared with oral irbesartan. This difference can only in part be explained by the 60% to 70% bioavailability of oral irbesartan. Studies with losartan have also demonstrated substantially less central effects after oral versus SC or IV administration. Actual pharmacokinetic data are not available, but one may speculate that more gradual and/or lower increases in plasma levels after oral administration result in less penetration across the blood-brain barrier compared with the rapid peaks in plasma levels achieved with IV or SC administration.

**Antihypertensive Effectiveness of Irbesartan in Dahl S on High Salt**

In previous studies, we showed that losartan at 1 mg · kg⁻¹ · d⁻¹ ICV fully prevents the sympathoexcitation and development of hypertension in Dahl S rats on high-salt intake. Similar results were obtained with the AT1 receptor blocker CV-11974. In the present study, ICV irbesartan only attenuated the hypertensive response to high-salt intake. Because at the infusion rates used pressor responses to ICV Ang II were only attenuated, taken together these studies suggest that ICV irbesartan at the infusion rates used only partially blocked the brain RAS and therefore only partially prevented the hypertension.

Oral irbesartan caused a high degree of peripheral AT1 receptor blockade, a moderate degree of central blockade, and an attenuation of the hypertensive response to high-salt intake. The latter 2 effects are rather similar to those caused by ICV irbesartan, whereas the extents of peripheral blockade by oral versus ICV irbesartan differed markedly. These findings are consistent with the concept that inhibition of the brain RAS by oral irbesartan resulted in blunting of the hypertension caused by high-salt intake in Dahl S rats.

The SC treatment with irbesartan at the highest dose caused at least as much peripheral AT1 receptor blockade as oral irbesartan. However, at this SC dose, the central block-
ade was more effective and the hypertension was fully prevented. The lower SC dose caused less peripheral blockade compared with oral irbesartan but a similar central blockade and similar attenuation of the hypertension induced by high-salt intake. 

In a comparison of the antihypertensive efficacy and the degree of central versus peripheral AT1 receptor blockade caused by irbesartan administered ICV, SC, or orally, it is clear that the degree of central blockade parallels the antihypertensive effect and not the degree of peripheral blockade. This finding is consistent with our previous study showing that the hypertension in Dahl S rats caused by high-salt intake depends on a functional brain RAS. The present study establishes that inhibition of the brain RAS can contribute to a significant extent to the therapeutic (ie, antihypertensive) effectiveness of chronic SC or oral treatment with AT1 receptor blockers such as irbesartan when administered in sufficiently high doses to cause central AT1 receptor blockade. The ratio of the doses required to inhibit the circulatory as well as brain RAS will likely vary among different AT1 receptor blockers related to their pharmacokinetic profile and extent of penetration through the blood-brain barrier. This finding likely also applies to other disease states in which the brain RAS contributes to sympathetic hyperactivity, such as congestive heart failure. Whether this concept also applies to humans remains to be established. However, the better outcome observed during the treatment of patients with congestive heart failure with high versus regular doses of ACE inhibitors may reflect an additional mechanism by high doses, such as inhibition of sympathetic outflow by central effects.

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**References**


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