Salt-sensitive hypertension develops after transient induction of ANG II-dependent hypertension in Cyp1a1-Ren2 transgenic rats

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Salt-sensitive hypertension develops after transient induction of ANG II-dependent hypertension in Cyp1a1-Ren2 transgenic rats. Systolic blood pressures were measured in conscious male Cyp1a1-Ren2 rats (Ren2 rats with salt-sensitive hypertension). This study was performed to determine whether a transient hypertensive episode can induce salt-sensitive hypertension in transgenic rats with inducible expression of the mouse Ren2 renin gene [strain name TGR(Cyp1a1-Ren2)]. Systolic blood pressures were measured in conscious male Cyp1a1-Ren2 rats (n = 6) during control conditions and during dietary administration of indole-3-carbinol (13C; 0.15%, wt/wt), for 14 days. Systolic pressure increased from 135 ± 5 to 233 ± 7 mmHg by day 14. I3C administration was terminated and blood pressure returned to normal levels (137 ± 5 mmHg) within 10 days. Subsequently, the rats were placed on a high-salt diet (8% NaCl) for 10 days. Systolic pressure increased by 34 ± 2 mmHg throughout 10 days of the high-salt diet. Neither glomerular filtration rate nor renal plasma flow was altered in Cyp1a1-Ren2 rats with salt-sensitive hypertension. In a separate group of male Cyp1a1-Ren2 rats (n = 6) transiently induced with 0.15% I3C for 14 days, administration of the superoxide dismutase mimetic tempol (4-hydroxy-2,2,6,6-tetramethyl piperidinoxyl, 2 mM) attenuated the increase in systolic pressure induced by high salt. Systolic pressure increased by only 11 ± 1 mmHg throughout 8 days of a high-salt diet and tempol administration. Thus transient induction of ANG II-dependent hypertension via activation of the Cyp1a1-Ren2 transgene induces salt-sensitive hypertension in these transgenic rats. The attenuation by tempol of the high salt-induced blood pressure elevation indicates that ANG II-induced production of superoxide anion contributes to the development of salt-sensitive hypertension after transient induction of ANG II-dependent hypertension.

ANG II-DEPENDENT HYPERTENSIVE rats can have even further increases in arterial pressure, when fed a high-salt diet (37). Additionally, transient exposure to ANG II can result in the development of persistent salt-sensitive hypertension in rats (14). The present study was performed to determine whether transient induction of ANG II-dependent hypertension results in salt-sensitive hypertension in transgenic rats [strain name TGR(Cyp1a1-Ren2)] with inducible expression of the mouse Ren2 renin gene. This transgenic rat line was generated by inserting a mouse Ren2 renin gene, fused to an 11.5-kb fragment of the cytochrome P-450 1a1 (Cyp1a1) promoter, into the genome of the Fischer 344 rat (12). Cyp1a1, which catalyzes the oxidation of a wide range of endogenous lipophilic compounds and xenobiotics (4, 7, 31), is not constitutively expressed but is highly inducible on exposure to various aryl hydrocarbons such as indole-3-carbinol (I3C) (4, 7, 8, 11, 15, 21, 31). Induction of Cyp1a1 is mediated by the aryl hydrocarbon receptor, which is a basic helix-loop-helix-transcription factor that binds to specific DNA elements in the Cyp1a1 promoter (4, 31). Therefore, rats transgenic for the Cyp1a1-Ren2 construct do not constitutively express the Ren2 renin gene. Rather, induction of the Cyp1a1 promoter by the dietary administration of an aryl hydrocarbon, such as I3C, results in the expression of the Ren2 renin gene. In this transgenic rat model, expression the Ren2 renin gene results in the development of ANG II-dependent hypertension (12). Hypertension is rapidly induced in a dose-dependent manner (12). Cessation of the administration of I3C results in the normalization of blood pressure, indicating the reversibility of the hypertension in this model (12). In essence, this model allows blood pressure to be increased using a benign and naturally occurring dietary supplement without the need for surgical intervention, dietary salt manipulation, or the administration of steroids.

The mechanism by which a transient ANG II-dependent hypertension results in salt-sensitive hypertension remains unclear. Reactive oxygen species, including superoxide anion (O2·−), are associated with hypertension and renal disease (16, 17). Oxidative stress has been identified in animal models, including Dahl salt-sensitive rats (10, 16, 17) and spontaneously hypertensive rats (29, 30). Moreover, it has been shown that ANG II stimulates superoxide anion production (22) and blockade of AT1 receptors attenuates oxidative stress (6). These findings suggest that superoxide anion contributes importantly to the pathogenesis of various forms of hypertension. Given the importance of oxidative stress in the pathogenesis of hypertension, it is possible that elevated levels of superoxide anions may contribute to the salt-sensitive hypertension that occurs following a transient episode of ANG II-dependent hypertension.

The superoxide dismutase (SOD) mimetic tempol (4-hydroxy-2,2,6,6-tetramethyl piperidinoxyl) (19, 24–26) protects many cells and tissues from oxidative stress (18, 27–30) and contributes to the salt-sensitive hypertension that occurs following transient induction of ANG II-dependent hypertension in Cyp1a1-Ren2 transgenic rats.
METHODS

Experiments were performed in adult male transgenic rats [TGR(Cyp1a1-Ren2)] with inducible expression of the Ren2 renin gene (12). All rats used in the present study were bred at the Tulane University School of Medicine from stock animals supplied from the University of Edinburgh, Edinburgh, UK. The experimental animals were divided into four groups. Group 1 (noninduced; n = 4) rats were maintained on a normal diet (1% NaCl, diet TD 90229, Harlan-Teklad, Madison, WI). Group 2 (noninduced + high-salt diet (HS); n = 5) normotensive rats were fed a high-salt diet (8% NaCl, diet TD 92012, Harlan-Teklad) for 9 days. Group 3 (induced + HS; n = 5) rats were fed a normal rat diet containing I3C (0.15%, wt/wt; diet TD 01060, Harlan-Teklad) for 14 days to induce ANG II-dependent hypertension. The rats were then placed on a normal non-I3C containing diet for 10 days, during which time the systolic pressures returned to normal levels. The rats were then fed a high-salt (8% NaCl) diet for 10–12 days. The rats were induced with 0.15% I3C for 15 days and then placed on a normal non-I3C containing diet for 12 days to allow the blood pressures to return to normal levels. Subsequently, the rats were fed a high-salt diet and treated with the SOD mimetic tempol (Sigma, St. Louis, MO). Tempol was added to the drinking water at a dose of 2 mM. This dose was used because it is similar to doses that have been previously shown to effectively reduce superoxide anion levels (9, 30).

Measurement of systolic blood pressure was obtained in conscious rats using tail-cuff plethysmography (ITC Instruments; Woodland Hills, CA). All rats were trained for 2 wk before the beginning of the experiment to habituate them to this procedure. Blood pressures were measured every 1–3 days throughout the duration of the study.

Renal clearance experiments were performed in all rats. For the clearance experiments, the rats were anesthetized with pentobarbital sodium (50 mg/kg iv). The rats were placed on a thermostatically controlled surgical table to maintain body temperature at 37°C. A tracheostomy was performed, and the animals were allowed to breathe air-enriched oxygen, which has been shown to improve the stability of arterial blood pressure of pentobarbital sodium-anesthetized rats (20). The left external jugular vein was cannulated with PE-50 tubing to allow intravenous infusion of solutions and additional doses of anesthetic. The left femoral artery was cannulated with PE-50 tubing to allow monitoring of arterial blood pressure. Blood pressure was monitored with a Statham pressure transducer (model P23 DC) and recorded using a computerized data-acquisition system (MP100 system; BIOPAC Systems, Santa Barbara, CA) with the Acqknowledge software package (version 3.2.4, BIOPAC). The rats were infused intravenously, at a rate of 1.2 ml/h, with isotonic saline containing 6% albumin (bovine; Calbiochem, San Diego, CA) during the surgery and thereafter with isotonic saline containing 1% albumin, 7.5% polyfructose (Inutest, Lentz, Austria), and 1.5% PAH (Merck, Whitehouse Station, NJ). A suprapubic incision was made, and the bladder was exposed by blunt dissection through the abdominal wall. The bladder was catheterized with PE-60 tubing to allow timed urine collections to be made. Following the surgery, the rats were allowed to stabilize for 1 h before the initiation of two 30-min urine collections. An arterial blood sample was obtained to allow determination of basal values of excretory function. At the end of each experiment, both kidneys were removed, decapsulated, blotted dry, and weighed.

Additional experiments were performed to determine the rate of urinary 8-isoprostane excretion, as a marker of oxidative stress. Experiments were performed in noninduced rats (n = 9), rats fed 0.15% I3C for 14 days, a normal diet for 10 days, followed by a high-salt diet for 10–12 days (n = 6), and rats fed 0.15% I3C for 15 days, a normal diet for 12 days, and then a high-salt diet plus tempol for 7–10 days (n = 6). The experimental procedure used was similar to that described above except that a single 30-min urine collection was obtained from anesthetized Cyp1a1-Ren2 rats for the determination of urinary 8-isoprostane excretion.

Urine volume was determined gravimetrically. Inulin and PAH concentrations in both urine and plasma were measured by standard spectrophotometry. Plasma and urine sodium and potassium concentrations were measured by flame photometry. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were estimated from the clearance of inulin and PAH, respectively. Urinary 8-isoprostane concentrations were measured using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Statistical analyses were performed using one-way ANOVA or one-way repeated-measures ANOVA followed by a Tukey test when appropriate. Statistical significance was defined as P < 0.05. All data are expressed as means ± SE.

RESULTS

The effects of dietary administration of I3C and high-salt intake on systolic blood pressures are summarized in Figs. 1 and 2. As shown in Fig. 1, chronic administration of I3C (0.15%, wt/wt) to group 3 (induced + HS) rats for 14 days induced a sustained increase in systolic blood pressure in Cyp1a1-Ren2 transgenic rats. Systolic blood pressure increased by day 1 (from 135 ± 5 to 174 ± 4 mmHg, P < 0.05) and increased to 233 ± 7 mmHg (P < 0.05) by day 14. Dietary administration of I3C was terminated, and systolic blood pressure returned to normal levels (137 ± 5 mmHg) during the following 10 days. Subsequently, the rats were placed on a high-salt diet (8% NaCl) for an additional 10 days. Systolic blood pressure increased to 159 ± 5 mmHg (P < 0.05) by day 1 and continued to increase to 171 ± 5 mmHg (P < 0.05) by day 10. Rats in group 4 (induced + HS + tempol) received the same treatment as group 3 except that the SOD mimetic tempol was administered at the same time as the high-salt diet. As previously described, tempol was added to the drinking water at a concentration of 2 mM. The systolic blood pressures of group 4 are shown in Fig. 2. Systolic blood pressure increased by day 1 (from 129 ± 2 to 154 ± 2 mmHg, P < 0.05) and increased to 206 ± 2 mmHg (P < 0.05) by day 15. Dietary administration of I3C was terminated, and systolic blood pressure returned to normal levels (126 ± 2 mmHg). Systolic blood pressure increased by only 11 ± 1 mmHg (from 126 ± 2 to 137 ± 5 mmHg, P < 0.05) throughout the 7–10 days of the high-salt diet and tempol administration. Thus tempol attenuated the increase in blood pressure caused by the administration of a high-salt diet following transient induction of ANG II-dependent hypertension. Group 2 rats (noninduced + HS) were fed a high-salt diet (8% NaCl) for 9 days but were not previously made hypertensive by dietary administration of I3C. Systolic blood pressure remained unaltered throughout 9 days of a high-salt (8% NaCl) diet. Systolic blood pressure averaged 132 ± 4 mmHg before the initiation of a high-salt diet and 119 ± 3 mmHg following 9 days of high-salt intake. Thus 9 days of a high-salt diet did not result in hypertension in noninduced normotensive Cyp1a1-Ren2 transgenic rats.

The mean arterial pressures (MAPs) obtained during the clearance experiments are shown in Fig. 3. The MAPs in group 2 were not different from those in the noninduced rats fed a normal-salt diet (group 1). Thus dietary administration of a high-salt diet (8% NaCl) did not induce an elevation of arterial pressure in normotensive Cyp1a1-Ren2 rats not previously induced with I3C. Previously induced rats fed a high-salt diet (group 3) had a MAP of 164 ± 5 mmHg, which was markedly
higher than the MAP of noninduced rats ($P < 0.05$). The administration of tempol attenuated the salt-induced elevations in MAP observed in transgenic rats that had been previously hypertensive. Previously induced rats that were fed a high-salt diet and administered tempol (group 4) had markedly a lower MAP compared with previously induced rats fed a high-salt diet (110 ± 8 vs. 164 ± 5 mmHg, $P < 0.05$).

The effects of each experimental protocol on GFR and RPF are shown in Figs. 4 and 5, respectively. Noninduced transgenic rats (group 1) had a mean GFR of $0.90.1$ ml/min/g kidney wt. A high-salt diet did not alter the GFR in noninduced rats (group 2) or transgenic rats that had been previously hypertensive as a result of the induction of the Ren2 renin gene (groups 3 and 4). In addition, the administration of tempol did not have an effect on GFR. The RPF in noninduced transgenic rats (group 1) was $3.5 ± 0.4$ ml/min/g kidney wt$^{-1}$. Neither noninduced transgenic rats (group 2) nor previously induced transgenic rats (groups 3 and 4) had an altered RPF in response to a high-salt diet. Even though the addition of tempol decreased the MAP systemically, tempol did not affect RPF.

The effects of a high-salt diet and a high-salt diet plus tempol on the absolute sodium excretion of noninduced and induced transgenic rats are shown in Fig. 6. All three groups fed a high-salt diet (groups 2–4) had an elevated sodium excretion compared with rats fed a normal-salt diet (group 1). Noninduced rats in group 2 fed a high-salt diet had significantly higher sodium excretion than noninduced rats fed a normal-salt diet ($5.1 ± 2.0$ vs. $0.6 ± 0.4 \mu$mol/min, $P < 0.05$). Previously induced rats fed a high-salt diet (group 3) had an absolute sodium excretion of $3.8 ± 0.7 \mu$mol/min ($P < 0.05$). The absolute sodium excretion of previously induced transgenic rats that were administered a high-salt and tempol was $2.2 ± 0.2 \mu$mol/min ($P < 0.05$).

The effects of a high-salt diet and high-salt diet plus tempol on 8-isoprostane excretion are shown in Fig. 7. Cyp1a1-Ren2 transgenic rats that were transiently induced and then fed a high-salt diet had markedly higher rates of excretion of urinary 8-isoprostane compared with noninduced control rats (34 ± 5

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**Fig. 1.** Systolic blood pressures of Cyp1a1-Ren2 transgenic rats fed a normal-salt diet containing 0.15% indole-3-carbinol (I3C) for 14 days, followed by a 10-day recovery period and then a high-salt diet (8% NaCl) for 10 days. *$P < 0.05$ compared with day 0. †$P < 0.05$ compared with before 8% NaCl.

**Fig. 2.** Systolic blood pressures of Cyp1a1-Ren2 TGR fed a normal-salt diet containing 0.15% I3C for 15 days, followed by a 12-day recovery period and then a high-salt diet+tempol (2 mM in the drinking water) for 7–10 days. *$P < 0.05$ compared with day 0. †$P < 0.05$ compared with before 8% NaCl.

**Fig. 3.** Effects of high-salt diet (HS) and HS+tempol on mean arterial pressures of noninduced and induced Cyp1a1-Ren2 transgenic rats. *$P < 0.05$ vs. noninduced. †$P < 0.05$ vs. other 3 groups.

**Fig. 4.** Effects of HS and HS+tempol on glomerular filtration rate of noninduced and induced Cyp1a1-Ren2 transgenic rats.
vs. 13 ± 5 pg·min⁻¹·g⁻¹, \( P \leq 0.01 \)). When tempol was administered together with a high-salt diet, urinary excretion of 8-isoprostane averaged 13 ± 4 pg·min⁻¹·g⁻¹ (\( P \leq 0.01 \) vs. previously induced rats fed a high-salt diet alone). Thus chronic administration of tempol attenuated the high salt-induced increase in 8-isoprostane excretion.

**DISCUSSION**

Previous investigations presented a new model demonstrating that short-term exposure to intravenous ANG II results in salt-sensitive hypertension (14, 23). In those studies, rats that had not been previously exposed to ANG II and fed a high-salt diet remained normotensive (14). However, rats that had been previously exposed to ANG II became hypertensive when placed on a high-salt diet (14). Those studies proposed that the transient exposure to ANG II caused functional changes in the kidney that prevented the previously hypertensive rats from adjusting to a salt load. The results of this study confirm the earlier findings. However, the present study is novel in that it demonstrates a new model of salt-sensitive hypertension using the Cyp1a1-Ren2 transgenic rat, where well-regulated ANG II-dependent hypertension can be induced without the need for exogenous ANG II administration. This transgenic rat line was generated by inserting a mouse Ren2 renin gene into the genome of the Fischer 344 rat (12). The Ren2 renin gene is induced by the administration of the aryl hydrocarbon I3C, resulting in the development of ANG II-dependent hypertension (12). This model is reversible, because the cessation of dietary I3C resulted in the normalization of blood pressure. Whereas rats, which had not been previously hypertensive and were fed a high-salt diet, remained normotensive, the administration of a high-salt diet, following the ANG II-dependent hypertensive episode, resulted in elevated blood pressure. This new model allows blood pressure to be increased using a dietary supplement without the need for surgical intervention. Therefore, this model of salt-sensitive hypertension is induced by a transient ANG II-dependent hypertensive episode caused by the induction of the Ren2 renin gene.

In the present study, neither GFR nor RPF was altered in Cyp1a1-Ren2 rats with salt-sensitive hypertension. This indicates that reductions in renal hemodynamic function were not primarily responsible for maintaining salt-sensitive hypertension in these rats. However, it should be recognized that renal hemodynamic function was not measured during the very early stages of salt-sensitive hypertension, and it is possible that reductions in renal hemodynamics may have contributed to the early increase in blood pressure induced by the high-salt diet. Nevertheless, the present observation that neither GFR nor RPF was altered in Cyp1a1-Ren2 rats during the maintenance phase of salt-sensitive hypertension suggests that enhanced tubular reabsorption contributes importantly to the maintenance of this form of salt-sensitive hypertension. In this regard, whereas Cyp1a1-Ren2 rats with salt-sensitive hypertension exhibited higher sodium excretion rates than normotensive Cyp1a1-Ren2 rats fed a normal-salt diet, the sodium excretion rates in Cyp1a1-Ren2 rats with salt-sensitive hypertension were not different from values measured in normotensive rats fed a high-salt diet. In essence, despite a markedly elevated
MAP, Cyp1a1-Ren2 rats with salt-sensitive hypertension exhibited an impaired sodium excretory response to the high salt-induced increase in arterial blood pressure. Such an impaired sodium excretory function likely reflects inappropriately elevated tubular reabsorption capability. Thus the present findings suggest that transient induction of ANG II-dependent hypertension causes a derangement in renal function that leads to enhanced tubular reabsorption and subsequent impairment of the ability of the kidney to respond to elevated salt intake. In this regard, a recent study found that a decrease in sodium excretion in Dahl salt-sensitive rats was due to increased tubular reabsorption (33). Another study reported that increased activity of the renal Na-K-Cl cotransporter NKCC2 contributes to the inability of Dahl salt-sensitive rats to excrete sodium (1). A previous study with salt-sensitive hypertension following ANG II-dependent hypertension showed that injury to peritubular capillaries and tubulointerstitium of the kidney and reduced function of endothelial nitric oxide (NO) synthase led to the reduced ability of the kidney to excrete a salt load (14). It should be recognized, however, that the present data do not allow assessment of the specific derangements in tubular transport function responsible for the overall augmentation of sodium reabsorptive capability of the kidney in this form of salt-sensitive hypertension. Further studies are required to address this issue.

With regard to the effects of tempol on the systolic pressure responses to high-salt intake, it should be emphasized that blood pressure was measured in conscious rats using tail-cuff plethysmography, which can influence stress in the rats. In view of this, and given that tempol has been shown to inhibit sympathetic tone (36), it is possible that the tempol-induced attenuation of salt-sensitive hypertension occurred, in part, as a result of a tempol-mediated decrease in sympathetic tone. However, in the present study rats with salt-sensitive hypertension had markedly increased urinary 8-isoprostanate excretion. Furthermore, chronic treatment with tempol prevented the high salt-induced increase in 8-isoprostanate excretion. Thus these data are consistent with the notion that the reduction of blood pressure in tempol-treated rats was due primarily to a decrease in superoxide anion activity. The finding that chronic administration of tempol markedly attenuated the magnitude of salt-sensitive hypertension following transient induction of ANG II-dependent hypertension, therefore, indicates an important role for superoxide anion in the development of this form of salt-sensitive hypertension.

Oxidative stress has been reported to play a role in both renal injury (34) and hypertension (13, 32). ANG II-dependent hypertension can increase superoxide production by activating the NADH/NADPH oxidase system (22). Increased superoxide levels can decrease the bioavailability of NO (35). Since NO is a vasodilator and promotes natriuresis (3), it is possible that increased levels of superoxide anion can promote salt sensitivity by decreasing the bioactivity of NO. Superoxide has also been shown to contribute to salt-sensitive hypertension by decreasing the bioavailability of 20-HETE which leads to an elevation in loop chloride transport in Dahl salt-sensitive rats (9). The importance of superoxide anion in the development of salt-sensitive hypertension has also been shown in studies that limited their actions. A recent study reported that the immunosuppressive drug mycophenolate mofetil was able to attenuate salt-sensitive hypertension following exposure to ANG II (23). This same study showed that mycophenolate mofetil decreased the number of superoxide-producing cells in rats previously exposed to ANG II. Vitamin E, which has antioxidant properties, has been shown to prevent renal and arterial injuries in Dahl salt-sensitive rats (2). In addition, superoxide is metabolized by SOD (35). Therefore, SOD is able to reduce the level of oxidative stress. The SOD mimetic tempol has been shown to attenuate the development of hypertension in Dahl salt-sensitive rats (9) and spontaneously hypertensive rats (29). It has also been reported that an intravenous infusion of tempol reduced the salt-induced increase in arterial pressure and decreased superoxide anion release in Dahl salt-sensitive rats (16). Thus the present study confirms the role of superoxide anion in mediating salt-sensitive hypertension and demonstrates the ability of the SOD mimetic tempol to markedly attenuate the development of salt-sensitive hypertension in Cyp1a1-Ren2 rats following a transient episode of ANG II-dependent hypertension.

In summary, the present study demonstrates a new model for salt-sensitive hypertension where a transient induction of ANG II-dependent hypertension via short-term activation of the Cyp1a1-Ren2 transgene induces salt-sensitive hypertension in these transgenic rats. The finding that neither GFR nor RPF was altered in rats with salt-sensitive hypertension suggests that enhanced tubular reabsorption contributes importantly to this form of hypertension. This is supported by the observation that rats with salt-sensitive hypertension had increased levels of sodium excretion, but at the expense of an increased arterial pressure. The attenuation by tempol of the high salt-induced blood pressure elevation indicates ANG II-induced production of superoxide anions contribute to the development of salt-sensitive hypertension after transient induction of ANG II-dependent hypertension.

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REFERENCES


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