The interrelationship between TGF-β1 and nitric oxide is altered in salt-sensitive hypertension

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Ying, Wei-Zhong, and Paul W. Sanders. The interrelationship between TGF-β1 and nitric oxide is altered in salt-sensitive hypertension. Am J Physiol Renal Physiol 285:F902–F908, 2003. First published July 15, 2003; 10.1152/ajprenal.00177.2003.—The study of salt-sensitive hypertension has been facilitated by development of genetic models, especially the Dahl/Rapp salt-sensitive (S) rat. S rats rapidly become hypertensive after initiation of a diet containing 8.0% NaCl and subsequently develop arteriolonephrosclerosis and renal failure, whereas the salt-resistant (R) strain remains normotensive on the same diet. The purpose of the present study was to use these strains to demonstrate the interactions between transforming growth factor-β1 (TGF-β1) and nitric oxide (NO). Young, male S and R rats were fed for 4 days diets that contained either 0.3 or 8.0% NaCl. An increase in dietary salt increased kinase activities of both p38 MAPK and p42/44 MAPK in cytoplasmic extracts from aortic rings and isolated glomeruli from both strains. Inhibition of either pathway with PD-098059 or SB-203580 decreased production of TGF-β1 and nitrate plus nitrite (NOx). In both strains, production of active TGF-β1 and NOx linearly correlated. Incubation of aortic rings and isolated glomeruli with the NO donor NOR3 decreased TGF-β1, whereas the NO synthase inhibitor N^ω-nitro-L-arginine methyl ester increased production. The inhibitory effect of NO on production of TGF-β1 was reduced in preparations from S rats. Although a close interrelationship existed between TGF-β1 and NO in both strains, production of TGF-β1 was increased in prehypertensive S rats and was further exaggerated with the increase in dietary salt intake. Augmented vascular and glomerular production of TGF-β1 and diminished NO may contribute to the development of hypertensive nephrosclerosis in S rats.

dietary salt; transforming growth factor-β1; salt sensitivity; Dahl/Rapp rat

Dietary salt intake varies in the population and modulates blood pressure (15, 22), although the significance of this effect has been questioned (1, 14). This longstanding debate, however, is mitigated by experiments that were performed in the 1950s and demonstrated that rats exposed to increasing amounts of NaCl in the diet experienced a dose-dependent reduction in life span related to the development of arteriosclerosis and chronic kidney disease (19). A more recent study (34) showed that a high-salt diet promoted cardiac and renal fibrosis in normotensive and hypertensive strains of rats and was associated with increased expression of transforming growth factor (TGF)-β, a fibrogenic growth factor that participates in production of extracellular matrix proteins. Several studies demonstrated that a high-salt intake exacerbates chronic kidney disease. In a rodent model of progressive renal disease, despite a lack of improvement in blood pressure, salt restriction proved to be more effective than diuretics alone at preventing glomerular sclerosis (3). An increased intake of salt was also associated with acceleration of progressive renal failure in patients with underlying chronic kidney disease (10). These findings suggest that dietary salt intake promotes cardiovascular-renal disease, particularly in susceptible populations.

Recent reports support the hypothesis that dietary salt has effects that are independent of blood pressure. A high-salt diet accelerated the progression of renal failure in a rodent model of chronic allograft nephropathy, without changing blood pressure (23). Aortic endothelium and glomeruli from normotensive Sprague-Dawley rats responded to an increase in dietary salt by increasing synthesis and release of TGF-β1 (29, 30, 32). The mechanism by which dietary salt activated the vascular endothelium was shown to be compatible with a shear effect, which increased the kinase activities of both p38 MAPK and p42/44 MAPK. Both pathways were required to produce TGF-β1 (31, 33). In turn, TGF-β1 directly stimulated nitric oxide (NO) production through NOS3, the endothelial isoform of NO synthase (29, 30). These effects occurred without a change in blood pressure. Thus, in the normal condition, an increase in dietary salt intake promotes the synthesis of TGF-β and subsequently NO in the kidney and arterial wall.

The purpose of the present series of experiments was to determine if dietary salt produced similar effects in isolated aortic rings and glomeruli from rats.
responses in salt-resistant and salt-sensitive rats and further determine if NO played an important feedback role in modulating production of TGF-β₁ under these conditions. Because of their consistent and reproducible responses to increased salt intake, the inbred strains of Dahl/Rapp rat provided ideal models with which to study salt resistance and salt sensitivity (21).

METHODS

Animal preparation. Studies were conducted by using 40 male Dahl/Rapp salt-sensitive (S) and 40 male Dahl/Rapp salt-resistant (R) rats, 28 days of age, obtained from Charles River Laboratories (Wilmington, MA). The rats were housed under standard conditions and fed formulated diets (AIN-76A, Dyets, Bethlehem, PA) that contained 0.3% (wt/wt) or 8.0% (wt/wt) NaCl. These diets were prepared specifically to be identical in protein composition and differed only in NaCl and sucrose content. This standardized protocol produced significant increases in blood pressure in S rats after 3 days on the 8.0% NaCl diet, whereas blood pressure of R rats remained unchanged (7). On day 4, the rats were anesthetized by 50 mg/kg body wt ip injection of pentobarbital sodium (Abbott Laboratories, North Chicago, IL). For studies involving MAPK, the aorta and kidneys were perfused in situ with a cold isotonic heparinized perfusion solution that contained (in mmol/l) 90 NaCl, 50 sodium fluoride, 1 Na₃VO₄, and 10 sodium pyrophosphate. Fifty milliliters of solution were perfused over 2 min. In the remaining experiments, the aorta and kidneys were perfused in situ with cold isotonic saline. The aorta and kidneys were harvested under sterile conditions.

Western blot analysis and kinase assays of p38 MAPK and p42/44 MAPK. Harvesting of aortic tissue, generation of protein lysates, and Western blotting proceeded as described previously (31, 33). The primary antibodies were diluted 1:1,000 and recognized specifically to be identical in protein composition and differed only in NaCl and sucrose content. This standardized protocol produced significant increases in blood pressure in S rats after 3 days on the 8.0% NaCl diet, whereas blood pressure of R rats remained unchanged (7). On day 4, the rats were anesthetized by 50 mg/kg body wt ip injection of pentobarbital sodium (Abbott Laboratories, North Chicago, IL). For studies involving MAPK, the aorta and kidneys were perfused in situ with a cold isotonic heparinized perfusion solution that contained (in mmol/l) 90 NaCl, 50 sodium fluoride, 1 Na₃VO₄, and 10 sodium pyrophosphate. Fifty milliliters of solution were perfused over 2 min. In the remaining experiments, the aorta and kidneys were perfused in situ with cold isotonic saline. The aorta and kidneys were harvested under sterile conditions.

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RESULTS

R and S rats fed 8.0% NaCl diet demonstrated increased serine phosphorylation state and kinase activity of both p42/44 MAPK and p38 MAPK in the aorta and glomeruli. Previous studies using the Sprague-Dawley rat, which is not salt sensitive, demonstrated that an increase in dietary salt intake increased the phosphorylation state and kinase activity of p42/44 MAPK and p38 MAPK (31, 33). The initial intent of the present studies was to determine if these pathways were activated in S rats during the development of salt-sensitive hypertension. Blood pressures of S rats have been shown consistently to increase within 4 days of a diet containing 8.0% NaCl, whereas R rats remained normotensive (7). With the use of cytoplasmic extracts of aortic tissue, compared with animals maintained on 0.3% NaCl diet, similar increases (P < 0.05) in the amount of protein that was phosphorylated and

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<th>p42/44 MAPK</th>
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Fig. 1. Increase in dietary NaCl increased phosphorylation and activity of p42/44 MAPK in aortas of both Dahl/Rapp salt-sensitive (S) and salt-resistant (R) rats (n = 4 rats in each group). Bottom: relative densities of the bands shown in the gels. Compared with animals on 0.3% NaCl, cytoplasmic extracts of aortic tissue from rats on 8.0% NaCl contained increased (P < 0.05) amounts of phospho-p42/44 MAPK and kinase activity of p42/44 MAPK. Total levels of p42/44 MAPK did not differ among the groups. *P < 0.05 compared with data obtained from the same strain of rat on the 0.3% NaCl diet.
enzyme activity of both p42/44 MAPK (Fig. 1) and p38 MAPK (Fig. 2) were demonstrated in S and R rats on 8.0% NaCl diet for 4 days. The same was found when cytoplasmic extracts from kidney cortex were used (data not shown). Kinase activities of p42/44 MAPK and p38 MAPK were also increased ($P < 0.05$) in cytoplasmic extracts from isolated glomeruli of S and R rats on 8.0% NaCl diet (Fig. 3).

Fig. 2. Increase in dietary NaCl increased phosphorylation and activity of p38 MAPK in aortas of both S and R rats ($n = 4$ rats in each group). Bottom: relative densities of the bands shown in the gels. Compared with animals on 0.3% NaCl, cytoplasmic extracts of aortic tissue from rats on 8.0% NaCl contained increased ($P < 0.05$) amounts of phospho-p38 MAPK and kinase activity of p38 MAPK. Total levels of p38 MAPK did not differ among the groups. $^*P < 0.05$ compared with data obtained from the same strain of rat on the 0.3% NaCl diet.

Fig. 3. Increase in dietary NaCl increased activities of both p38 MAPK and p42/44 MAPK in glomeruli of S and R rats ($n = 4$ rats in each group). Bottom: relative densities of the bands shown in the gels. Compared with animals on 0.3% NaCl, cytoplasmic extracts of glomeruli from rats on 8.0% NaCl demonstrated increased ($P < 0.05$) activities of both p38 MAPK and p42/44 MAPK. $^*P < 0.05$ compared with data obtained from the same strain of rat on the 0.3% NaCl diet.
p42/44 MAPK and p38 MAPK inhibitors decreased production of total and active TGF-\(\beta_1\) and \(\text{NO}_x\) by aortic tissue from S and R rats. Aortic rings were incubated in RPMI overnight, and medium was used to determine production of total and active TGF-\(\beta_1\) and \(\text{NO}_x\) (Fig. 4). With an increase in dietary salt intake, aortic rings from both S and R rats increased \((P < 0.05)\) production of total and active TGF-\(\beta_1\), as well as \(\text{NO}_x\), compared with ring preparations from S and R rats on 0.3% NaCl diet. Compared with aortic ring preparations from R rats, however, tissue from S rats demonstrated an exaggerated \((P < 0.05)\) production of total and active TGF-\(\beta_1\) and diminished \((P < 0.05)\) production of \(\text{NO}_x\) with the increase in dietary salt. Consistent with previous findings in Sprague-Dawley rats (31, 33), addition of specific, cell-permeable inhibitors of p42/44 MAPK (PD-098059) and p38 MAPK (SB-203580) diminished production of TGF-\(\beta_1\) and \(\text{NO}_x\). Also, consistent with previous studies (31, 33) was the observation that the inhibitory effects occurred in preparations from rats on the 0.3% NaCl diet, suggesting a role for p38 MAPK and p42/44 MAPK in the baseline regulation of TGF-\(\beta_1\) in both strains. Mechanical denudation of the endothelium of aortic rings from R and S rats on 0.3 and 8.0% NaCl diets reduced \((P < 0.05)\) production of total and active TGF-\(\beta_1\), compared with rings that had intact endothelia from the same animals on the same diet. Endothelial denudation also decreased NO production to low levels that did not differ \((P < 0.05)\) among the groups.

Production of active TGF-\(\beta_1\) and \(\text{NO}_x\) by aortic rings directly correlated. With the use of data from the aortic ring preparations, a direct correlation between active TGF-\(\beta_1\) and \(\text{NO}_x\) was observed both in S \((r^2 = 0.794; P < 0.05)\) and R \((r^2 = 0.749; P < 0.05)\) rats (Fig. 5). A distinct separation of the curves was apparent, such that at any level of \(\text{NO}_x\) production, active TGF-\(\beta_1\) production was greater in aortic rings from S rats.

Production of total and active TGF-\(\beta_1\) was modulated by \(\text{NO}_x\) production. To determine if NO directly affected production of TGF-\(\beta_1\), preparations of aortic rings were incubated overnight in medium that contained the NO synthase inhibitor L-NAME (100 \(\mu\)M), the NO donor NOR3 (10 \(\mu\)M), and vehicle alone. In aortic ring preparations from both R and S rats on 0.3 and 8.0% NaCl diets, addition of L-NAME increased \((P < 0.05)\) and NOR3 decreased \((P < 0.05)\) production of total and active TGF-\(\beta_1\); the expected
changes in NOx production in response to addition of these chemicals to the medium were demonstrated (Fig. 6). In these experiments where NO levels were manipulated, NOx levels correlated inversely with total TGF-β1 levels in both R (r² = 0.898; P < 0.05) and S (r² = 0.879; P < 0.05) rats. The experiment was repeated using preparations of isolated glomeruli with a similar effect of NO on TGF-β1 observed; i.e., compared with vehicle-treated preparations, addition of 100 μM Nω-nitro-l-arginine methyl ester (L-NAME) increased (P < 0.05) and 10 μM NOR3 decreased production of total and active TGF-β1. NOx accumulation was inhibited by L-NAME and was increased by NOR3. *P < 0.05 compared with the L-NAME- and NOR3-treated groups from the same strain on the same diet; †P < 0.05 compared with other strain on the same diet.

Fig. 6. Effect of alteration of NO levels was studied using aortic ring preparations from S and R rats on the 2 diets (n = 4 rats in each group). Top: results using tissue from the animals on 8.0% NaCl. Bottom: results from animals on 0.3% NaCl. In S and R rats on both diets, compared with vehicle-treated rings, addition of 100 μM Nω-nitro-l-arginine methyl ester (L-NAME) increased (P < 0.05) and 10 μM NOR3 decreased production of total and active TGF-β1. NOx accumulation was inhibited by L-NAME and was increased by NOR3. *P < 0.05 compared with the L-NAME- and NOR3-treated groups from the same strain on the same diet; †P < 0.05 compared with other strain on the same diet.

Fig. 7. Effect of alteration of NO levels was studied using isolated glomeruli from S and R rats on the 2 diets (n = 4 rats in each group). Under these experimental conditions, it should be noted that the glomerular preparations produced significantly more TGF-β1 and NOx than the aortic ring preparations. Top: results using tissue from the animals on 8.0% NaCl. Bottom: results from animals on 0.3% NaCl. In S and R rats on both diets, compared with vehicle-treated glomerular preparations, addition of 100 μM L-NAME increased (P < 0.05) and 10 μM NOR3 decreased production of total and active TGF-β1. NOx accumulation was inhibited by L-NAME and was increased by NOR3. *P < 0.05 compared with the L-NAME- and NOR3-treated groups from the same strain on the same diet; †P < 0.05 compared with other strain on the same diet.
DISCUSSION

Dietary salt intake has been shown to modulate the function of vascular endothelium and kidney, promoting expression of TGF-β1 and production of NO through NOS3 (29–33). The intent of the present study was to determine in further detail the nature of the relationship between TGF-β1 and NO under physiological conditions and during the development of salt-sensitive hypertension. Prior studies demonstrated that R rats remained normotensive during this protocol, whereas blood pressure of S rats increased by day 3 on the 8.0% NaCl diet and, by day 4 of the high-salt diet, mean blood pressure averaged 129 ± 1 mmHg. S rats maintained on 0.3% NaCl manifested no increases in blood pressure (7). Continuing the high-salt diet promoted further increases in blood pressure in S rats and, after 2 wk, end-organ damage in the form of renal failure ensued and became severe and irreversible after 3 wk (9). The consistency of the blood pressure response made S rats an ideal model to investigate the causes and consequences of salt-induced hypertension. New findings of the present study included: 1) an increase in dietary salt produced comparable increases in the activities of p38 MAPK and p42/44 MAPK in the aortas and glomeruli of S and R rats; 2) in both strains, the increased expression of TGF-β1 and NO was dependent on the coordinated activation of p38 MAPK and p42/44 MAPK; 3) production of TGF-β1 and NO was tightly interrelated; and 4) production of TGF-β1 was higher and NO diminished in aortas of prehypertensive S rats compared with R rats; these differences were accentuated by the high-NaCl intake in the S rats and did not appear to be associated with alteration of activation of either the p38 or p42/44 MAPK pathways. Mechanical denudation of aortic ring preparations demonstrated that endothelial cells were the predominant source of both TGF-β1 and NO. The data suggested that young, prehypertensive S rats possessed an underlying disorder of endothelial cell function that was exacerbated by the development of hypertension induced by an increase in dietary salt intake.

The findings emphasized the role that dietary salt may play in modulating TGF-β1 production under physiological and pathological conditions. The physiological response to an increase in dietary salt consisted of increased production of TGF-β1, which increased NO production through NOS3 (29, 30). In turn, the increase in NO production provided a feedback inhibition of TGF-β1. Thus, in the physiological setting, NO mitigated the effects of TGF-β1. Interaction between TGF-β1 and NO has also been seen in pathological conditions, including cyclosporine-associated nephropathy induced in vivo in the rat (25) and glucose-mediated perturbation of TGF-β1 and NO production by mesangial cells in culture (11, 27). Compared with R rats, despite the persistence of feedback inhibition by NO, a significant increase in active TGF-β1 production and impaired release of NO by the arterial endothelium and glomeruli were observed in S rats. The importance of the present findings was underscored by experiments that demonstrated abnormal expansion of the mesangium and accumulation of extracellular matrix proteins in the pregglomerular arterioles of kidneys from hypertensive S rats (9). TGF-β1 is a fibrogenic growth factor that participates in the development of renal failure in a variety of disease processes (4, 5, 17, 18, 20, 24–26, 28, 35). Systemic administration of an antibody that neutralized TGF-β diminished renal injury and glomerulosclerosis in S rats; blood pressure improved slightly presumably because of better preservation of renal function (12). Increasing NO production in vivo also prevented the development of progressive renal failure in S rats (7–9). Thus the combined findings showed impaired regulation of TGF-β1, in part, because of diminished release of NO, promoted the accumulation of matrix material in the arterioles and glomeruli, and contributed to the subsequent development of nephrosclerosis in this model of salt-sensitive hypertension.

DISCLOSURES

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REFERENCES


