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Connecting tubule glomerular feedback (CTGF) in Hypertension

Hong Wang, M.D.[1], **Martin A. D'Ambrosio, M.D.**[1], **Jeffrey L. Garvin, Ph.D.**[1,2], **Yilin Ren, M.D.**[1], and **Oscar A. Carretero, M.D.**[1]

[1]Hypertension and Vascular Research Division, Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan

[2]Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio

Abstract

In Dahl salt-sensitive rats (Dahl SS), glomerular capillary pressure (P_{GC}) increases in response to high salt intake and this is accompanied by significant glomerular injury compared to spontaneously hypertensive rats (SHR) with similar blood pressure. P_{GC} is controlled mainly by afferent arteriolar (Af-Art) resistance, which is regulated by the vasoconstrictor tubuloglomerular feedback (TGF) and the vasodilator connecting tubule glomerular feedback (CTGF). We hypothesized that Dahl SS have a decreased TGF response and enhanced TGF resetting compared to SHR, and that these differences are due in part to an increase in CTGF. *In vivo*, using micropuncture we measured stop-flow pressure ($P_{SF,}$ a surrogate of P_{GC}). TGF was calculated as the maximal decrease in P_{SF} caused by increasing nephron perfusion, TGF resetting as the attenuation in TGF induced by high salt diet, and CTGF as the difference in TGF response before and during CTGF inhibition with benzamil. Compared to SHR, Dahl SS had 1) lower TGF responses in normal (6.6±0.1 *vs*. 11.0±0.2 mm Hg; $P<0.001$) and high-salt diets (3.3±0.1 *vs*. 10.1±0.3 mmHg; $P<0.001$), 2) greater TGF resetting (3.3±0.1 *vs*. 1.0±0.3 mmHg; $P<0.001$), and 3) greater CTGF (3.4±0.4 *vs*. 1.2±0.1 mmHg; $P<0.001$). We conclude that Dahl SS have lower TGF and greater CTGF than SHR, and that CTGF antagonizes TGF. Furthermore, CTGF is enhanced by a high-salt diet and contributes significantly to TGF resetting. Our findings may explain in part the increase in vasodilatation, P_{GC}, and glomerular damage in salt-sensitive hypertension during high salt intake.

Keywords

Dahl salt-sensitive; Spontaneously Hypertensive Rats; CTGF; TGF; stop-flow pressure; benzamil; salt-resistant

Introduction

There is evidence that in hypertension, glomerular capillary pressure (P_{GC}) greatly influences the progression of renal nephrosclerosis [1,2]. In African-Americans with salt-sensitive hypertension, high salt intake causes an abnormal renal hemodynamic response and an increase in estimated P_{GC}[3]. In Dahl salt-sensitive rats (Dahl SS), P_{GC} increases in

Corresponding author: Oscar A. Carretero, M.D., Hypertension and Vascular Research Division, Henry Ford Hospital, 2799 West Grand Blvd., Detroit, MI 48202, Phone: (313) 916-5236, Fax: (313) 916-1479, ocarret1@hfhs.org.

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response to high salt intake and this is accompanied by significantly greater glomerular injury compared to spontaneously hypertensive rats (SHR) with similar blood pressure [4-6]. P_{GC} is controlled by both afferent (Af-Art) and efferent arteriolar resistance. Af-Art resistance is regulated by mechanisms similar to other arterioles, including sympathetic nerve activity, angiotensin II, nitric oxide, eicosanoids, and myogenic response. In addition, Af-Art resistance is also regulated by two intrinsic renal autoregulatory mechanisms, namely tubuloglomerular feedback (TGF) and connecting tubule glomerular feedback (CTGF). TGF is initiated by increases in NaCl in the macula densa and causes Af-Art constriction, while CTGF is initiated by increases in NaCl in the connecting tubule and causes Af-Art dilatation [7] (see Figure 1). CTGF is initiated by Na entry *via* the epithelial Na channel (ENaC) in the connecting tubule and is blocked by the ENaC inhibitor benzamil. CTGF is mediated by prostaglandin E_2 and epoxyeicosatrienoic acids [8-11].

During high salt intake, if TGF were to remain unchanged, it would cause a decrease in P_{GC} and glomerular filtration due to enhanced distal delivery of NaCl, and thus decrease the renal natriuretic response to high salt intake. However, this does not occur because TGF resets, so that a greater amount of NaCl is required to elicit the same vasoconstriction [12]. In addition to high salt, TGF resetting occurs in response to physiological and pathophysiological conditions such as volume expansion, diabetes, and unilateral nephrectomy [13-16]. The mechanisms that mediate TGF resetting are not completely understood.

In vivo NaCl in the lumen of the distal nephron regulates Af-Art resistance *via* the combined effect of TGF and CTGF [10]. Thus, the observation that TGF is attenuated or reset in certain conditions could reflect an increase in CTGF that counteracts TGF. In fact, we have recently reported that CTGF partly mediates acute TGF resetting induced by sustained perfusion of single nephrons at the high end of the physiological tubular flow range [17].

The roles of TGF resetting and CTGF in hypertensive rats have not been well characterized. Here we studied for the first time the role of CTGF in the regulation of P_{GC} and salt-induced TGF resetting in Dahl SS and SHR. We hypothesized that Dahl SS have a decreased TGF response and enhanced TGF resetting compared to SHR, and that these differences are due in part to an increase in CTGF. To test this hypothesis, we used Dahl SS, Dahl salt resistant rats (Dahl SR), SHR and Wistar Kyoto rats (WKY) fed a normal or high-salt diet and performed micropuncture of individual nephrons to measure stop-flow pressure ($P_{SF,}$ a surrogate of P_{GC}). TGF was calculated as the decrease in P_{SF} caused by increasing nephron perfusion.

Methods

Male Dahl SS, Dahl SR, SHR, and WKY weighing 307.9 ± 2.6 g were fed either a normal (0.23% NaCl) or high salt diet (4% NaCl) for two weeks. In micropuncture experiments *in vivo*, two consecutive stop-flow pressure (P_{SF}) curves were performed. In half of the experiments, the ENaC blocker benzamil was added during the second P_{SF} curve to inhibit CTGF. TGF was calculated as the maximal decrease in P_{SF} caused by increasing nephron perfusion, TGF resetting as the attenuation in TGF induced by high salt diet, and CTGF as the difference in TGF response before and during CTGF inhibition with benzamil. An expanded Methods section is available online at http://hyper.ahajournals.org.

Results

1) Dahl SS and Dahl SR fed normal salt diet: TGF response and role of CTGF

Dahl SS (black circles) had an attenuated TGF response compared to Dahl SR (white circles). These differences reached statistical significance when the tubules were perfused at a rate of 20 nL/min or greater (Figure 2). In rats fed a normal salt diet, blocking CTGF potentiated the TGF response in both Dahl SR and Dahl SS (Figures 3A and 3B). Although this potentiation was somewhat greater in Dahl SS, it was not of statistical significance (Figure 3C). In time control experiments we confirmed that two consecutive TGF responses were reproducible in Dahl SR and Dahl SS with no time effect (see supplemental figures S1A and S1B).

SHR and WKY fed normal salt diet: TGF response and role of CTGF—SHR (black circles) had a greater TGF response compared to WKY (white circles). These differences reached statistical significance when the tubules were perfused at a rate of 20 nL/min or greater (Figure 4A). These data were normalized to baseline P_{SF} since basal pressure was significantly higher in the SHR (see absolute numbers in Figure 4B). Inhibition of CTGF with benzamil potentiated TGF response in WKY when the tubules were perfused at a rate of 30 nL/min or greater (Figure 5A). However, in SHR inhibition of CTGF did not potentiate the TGF response (Figure 5B), suggesting that SHR fed a normal salt diet have little or no CTGF. WKY tended to have greater CTGF than SHR ($P < 0.01$ for the overall ANOVA group comparison). When CTGF in SHR was compared to that of WKY at each individual flow rate, P values were < 0.05 at 30 and 40 nL/min, however, these differences did not reach statistical significance after adjustment for multiple comparisons (Figure 5C). In time control experiments we confirmed that two consecutive TGF responses were reproducible in WKY and SHR with no time effect (see supplemental figures S2A and S2B).

2) Dahl SS and Dahl SR: TGF resetting induced by high-salt diet (two weeks), role of CTGF in TGF resetting

When the rats were fed a high-salt diet (4% NaCl), TGF responses were attenuated in both Dahl SR and Dahl SS. These differences reached statistical significance when the tubules were perfused at 30 and 40 nL/min (Figure 6, panels A and B). However, the resetting was greater in Dahl SS than in Dahl SR (Figure 6C). Inhibition of CTGF with benzamil in Dahl SR and Dahl SS fed a high-salt diet led to a potentiation of TGF responses when the tubules were perfused at 30 and 40 nL/min (Figure 7, panels A and B). An inter-strain comparison showed that when fed a high-salt diet, Dahl SS had a greater CTGF response than Dahl SR (Figure 7C). In Dahl SS, the percentage of resetting due to CTGF was 53%, while in Dahl SR it was only 21% (supplemental Figure S3).

3) SHR and WKY: TGF resetting induced by high-salt diet (two weeks), role of CTGF in TGF resetting

When WKY rats were fed a high-salt diet, TGF responses were attenuated, these differences reached statistical significance when the tubules were perfused at 30 and 40 nL/min (Figure 8A). When SHR were fed a high-salt diet, there was a small decrease in TGF response but it did not reach statistical significance (Figure 8B). Resetting was significantly greater in WKY than in SHR (Figure 8C). Inhibition of CTGF with benzamil in WKY and SHR fed a high-salt diet led to potentiation of TGF responses when the tubules were perfused at 30 and 40 nL/min (Figure 9, panels A and B). An inter-strain comparison showed that when fed a high-salt diet, the CTGF response was greater in WKY than SHR (Figure 9C).

4) Dahl SS and SHR on normal and high-salt diet: Comparison of TGF response, TGF resetting and role of CTGF

Dahl SS rats on a normal salt diet had a significantly lower TGF response than SHR on normal salt diet (Figure 10, white circles *vs*. white triangles). TGF resetting induced by high salt intake was significantly greater in Dahl SS than in SHR (3.3 ± 0.1 *vs*. 1.0 ± 0.3 mm Hg; $P < 0.001$, Figure 10, delta between white and black circles *vs*. delta between white and black triangles). Inhibition of CTGF with benzamil potentiated TGF in Dahl SS to a greater extent than in SHR (3.4 ± 0.4 *vs*. 1.2 ± 0.1 mm Hg; $P < 0.001$, Figure 11, delta between black and white circles *vs*. delta between black and white triangles). These data suggest that TGF responses are much lower in Dahl SS than in SHR and that TGF resetting and the role of CTGF are more pronounced in Dahl SS than in SHR.

Discussion

In humans, susceptibility to hypertension-induced renal damage varies, with African-Americans at high risk [18]. African-Americans often have salt-sensitive hypertension [19], and high salt intake causes an abnormal renal hemodynamic response and an increase in estimated P_{GC}[3]. Thus their enhanced susceptibility to renal damage may be related to the increased P_{GC} associated with salt sensitivity, as salt sensitivity in humans predicts higher microalbuminuria in the short term [20] and higher mortality on long-term follow-up studies [21].

In animal models, there is substantial evidence that in hypertension P_{GC} greatly influences the progression of renal nephrosclerosis [1,2,4,22]. It is well known that at similar levels of systemic hypertension, Dahl SS but not SHR develop glomerular injury. Furthermore, these differences in glomerular pathology occur because Dahl SS but not SHR develop glomerular hypertension [4]. In SHR, glomerular capillary pressure in cortical nephrons is normal in spite of severe systemic hypertension because of a marked increase in preglomerular arteriolar resistance [5]. Thus glomeruli of SHR are protected from systemic hypertension. On the other hand, hypertensive Dahl SS display increased glomerular blood flow and glomerular capillary pressure due to decreased preglomerular arteriolar resistance [6]. The present study is the first to explore whether differences in glomerular hemodynamics between these strains may be due to differences in CTGF.

TGF responses in Dahl rats have not been well characterized. Wilcox and Welch reported that Dahl SS fed a low-salt diet had an attenuated TGF response compared to Sprague-Dawley rats [23]. On the other hand, Karlsen *et al*. found no differences in TGF between Dahl SS and Sprague-Dawley rats [24]. We have previously shown that Sprague-Dawley rats have CTGF, that CTGF antagonizes TGF, and that CTGF at least partially mediates TGF resetting induced acutely by volume expansion [10,17]. In the current work, we have not included Sprague-Dawley rats; rather, we have compared the Dahl SS to its genetic control and to SHR (to contrast these two models of hypertension). We believe our current work will help clarify the TGF response in Dahl rats, as well as provide the first studies of CTGF in hypertension. In our study, Dahl SS had an attenuated TGF response compared to Dahl SR on both normal and high-salt diets. Dahl SS fed a high-salt diet had greater TGF resetting and greater CTGF than Dahl SR. In Dahl SS inhibition of CTGF decreased TGF resetting by 53 % while in Dahl SR it did so by only 21%. Collectively, these results suggest that in Dahl SS fed a high-salt diet, CTGF is increased, leading to higher P_{SF} and P_{GC}. In this way, higher CTGF may participate in the development of nephrosclerosis. In this study, we used P_{SF} as a surrogate for P_{GC} since Dahl SS do not have superficial glomeruli that can be directly punctured to measure P_{GC} [25].

In contrast to Dahl SS, we found that SHR fed a normal salt diet have a much greater TGF response than WKY or Dahl SS and that TGF resetting induced by high salt intake was minimal or nonexistent, consistent with previous reports [26,27]. Furthermore, in SHR antagonism of CTGF to the TGF response and its contribution to TGF resetting were also minimal, indicating that a diminished CTGF may at least partially explain the enhancement in TGF and reduced TGF resetting. These data suggest that a decrease in CTGF in SHR increases preglomerular vascular resistance and helps maintain a normal P_{GC}, thus preventing renal damage, in stark contrast to Dahl SS.

Our findings in WKY also expand our understanding of the role of CTGF in TGF resetting in normotensive rats. We previously reported that CTGF partly mediates TGF resetting in normotensive Sprague-Dawley rats induced acutely by sustained perfusion of the nephron at a high flow rate for 30 minutes [17]. Here we found for the first time that CTGF also partly mediates TGF resetting that was induced chronically by high salt intake over two weeks. However, CTGF does not completely explain TGF resetting, as some TGF resetting still occurred even when CTGF was blocked. Likely, this represents intrinsic mechanisms of the macula densa that reset TGF, as previously described [12].

The mechanism by which CTGF is enhanced in Dahl SS remains unknown, but may relate to the fact that CTGF is initiated by Na transport in the CNT *via* ENaC [8]. In spite of their high blood pressure and low serum aldosterone levels, Dahl SS fed high salt have increased ENaC mRNA, protein, and sodium transport compared to Dahl SR [28-33]. Furthermore, Pavlov *et al*. recently measured ENaC single-channel activity by patch clamp studies in split-open cortical collecting ducts and found higher ENaC activity in Dahl SS on a high-salt diet compared to either a normal diet or to Dahl SR [34]. In addition, CTGF is mediated by prostaglandin E_2 (PGE_2) and epoxyeicosatrienoic acids (EETs) [9,11]. In Dahl SS, high salt intake increases renal cortical COX-2 expression [35] and urinary PGE_2 excretion [36]. Thus, it is possible that in Dahl SS, increased ENaC and PGE_2 may cause enhanced CTGF, enhanced P_{GC}, and enhanced glomerular damage. Conversely, attenuation of CTGF in SHR may be due to decreased EETs, since soluble epoxide hydrolase, the enzyme that metabolizes EETs, is increased in the kidney in this strain [37]. Therefore, decreased levels of EETs, which partly mediate CTGF, could explain the decrease in CTGF in SHR.

In summary, our studies provide direct evidence that a high-salt diet causes TGF resetting, and that CTGF mediates TGF resetting induced by a high-salt diet, at least in part. Hypertensive Dahl SS have lower TGF compared to either SHR or Dahl SR, and because of an increased TGF resetting in Dahl SS these differences become exaggerated on a high-salt diet. These differences are due in part to a greater CTGF in Dahl SS fed a normal salt diet, as well as greater enhancement of CTGF by a high-salt diet. Our findings may help explain the excessive increase in P_{GC} and glomerular damage observed in salt-sensitive hypertension.

Perspectives

An increase in CTGF may explain the higher glomerular pressure and renal damage in salt-sensitive hypertensive individuals, such as African-Americans, the elderly, and the diabetic. ENaC-blocking drugs (potassium-sparing diuretics), by blocking CTGF and decrease glomerular perfusion pressure, could be useful in preventing hypertensive nephrosclerosis. Our studies may also help explain the beneficial effects seen with mineralocorticoid receptor blockers and suggest new targets for prevention of renal damage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

What is New?

- CTGF is a novel mechanism of regulation of afferent arteriole resistance.
- We show that CTGF is responsible for part of the TGF resetting induced by chronic high salt intake.
- In salt-sensitive hypertension, CTGF is augmented and explains more than 50% of TGF resetting.

What is Relevant?

In the United States, over one fourth of adults diagnosed with hypertension have moderate to severe chronic kidney disease, and hypertension is the second leading cause of end-stage renal disease (ESRD). In the last two decades, the incidence of hypertension-induced ESRD has nearly doubled, even as control of hypertension increased from 24% to 50%. Despite adequate blood pressure control, renal function declines more often in individuals with salt-sensitive hypertension, including African-Americans, the elderly, and people with diabetes mellitus, all of whom are more susceptible to developing hypertensive renal damage. The cause of this enhanced susceptibility is related to the increased P_{GC} associated with salt-sensitivity, as salt-sensitivity in humans predicts higher microalbuminuria in the short term and higher mortality on long-term follow-up studies.

Summary

Our study shows that CTGF, which is a novel mechanism of regulation of afferent arteriole resistance, is responsible for more than 50% of the increase in P_{GC} in salt-sensitive hypertension. Understanding the mechanism that increases P_{GC} in salt-sensitive hypertension may lead to both prevention and better treatment of renal disease in salt-sensitive hypertension.

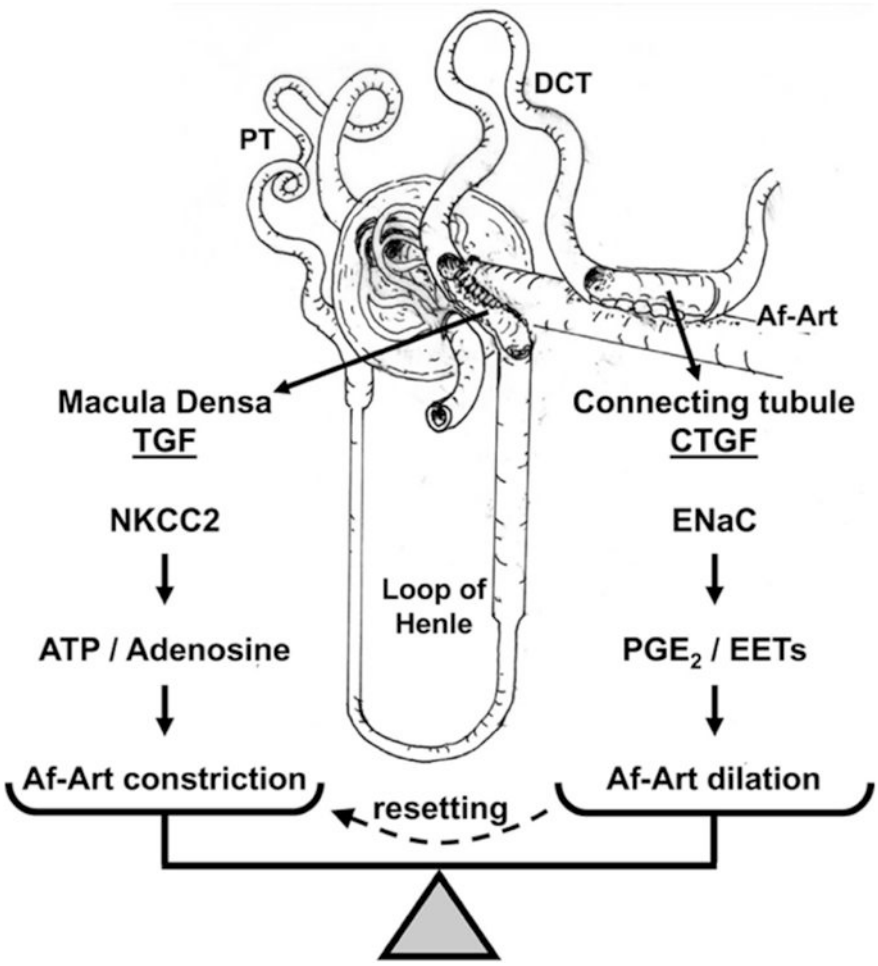

Figure 1. Schematic representation of TGF and CTGF

The macula densa triggers TGF when Na is reabsorbed via the Na/K/2Cl cotransporter type 2 (NKCC2), by releasing ATP which is broken down to adenosine, which in turn causes constriction of the Af-Art. The connecting tubule triggers CTGF when Na is reabsorbed via the epithelial sodium channel (ENaC), by releasing epoxyeicosatrienoic acids (EETs) and prostaglandin E2 (PGE2), which cause dilation of the Af-Art. PT indicates proximal tubule, and DCT distal convoluted tubule.

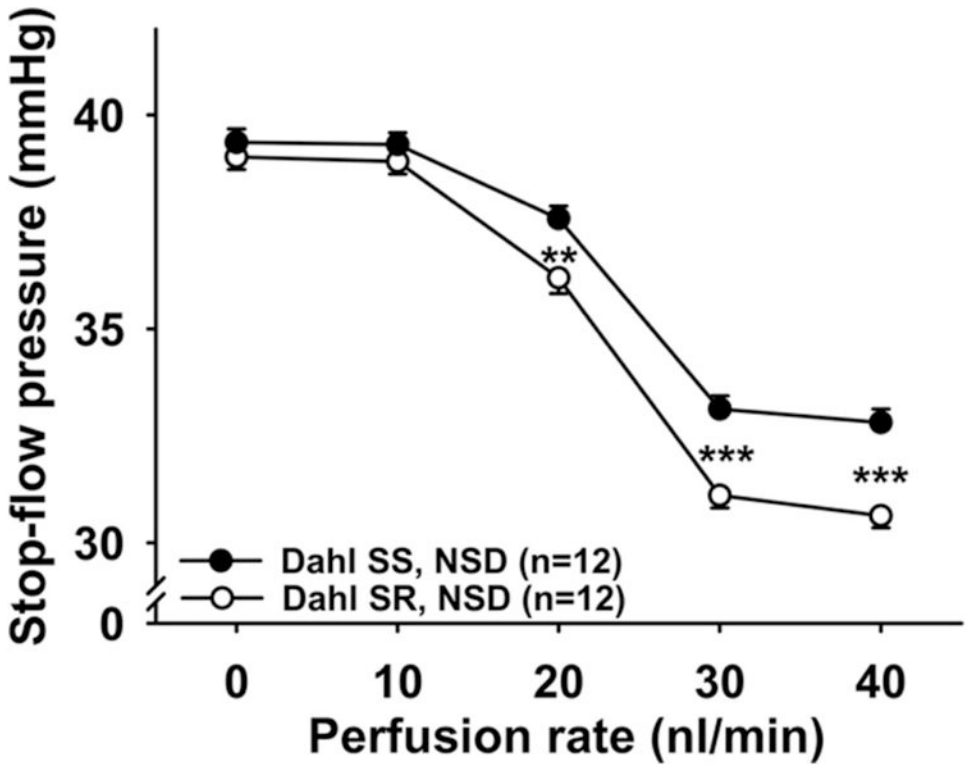

Figure 2. TGF response in Dahl SR and Dahl SS fed normal salt diet

TGF induced by increased perfusion rates in the late proximal tubule in Dahl SR (○) and Dahl SS (●) on a normal salt diet (NSD). When the tubules were perfused at 20, 30, and 40 nL/min, TGF was significantly attenuated in Dahl SS. ** $P < 0.01$, *** $P < 0.001$, Dahl SR *vs*. Dahl SS.

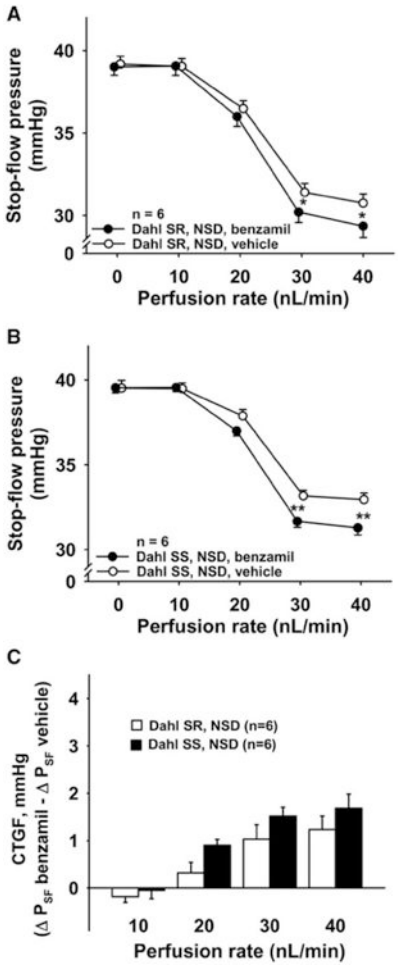

Figure 3. Role of CTGF in TGF response in Dahl SR and Dahl SS fed normal salt diet

In rats fed a normal salt diet (NSD), two TGF responses were elicited, first in the presence of CTGF (○, vehicle), then during inhibition of CTGF (●, benzamil 1μM). Inhibition of CTGF potentiated the TGF response in both Dahl SR (Panel A) and Dahl SS (Panel B). Panel C: Both Dahl SR (open bars) and Dahl SS (closed bars) showed CTGF, but there were no differences between strains. * $P < 0.05$, ** $P < 0.01$, vehicle *vs*. benzamil.

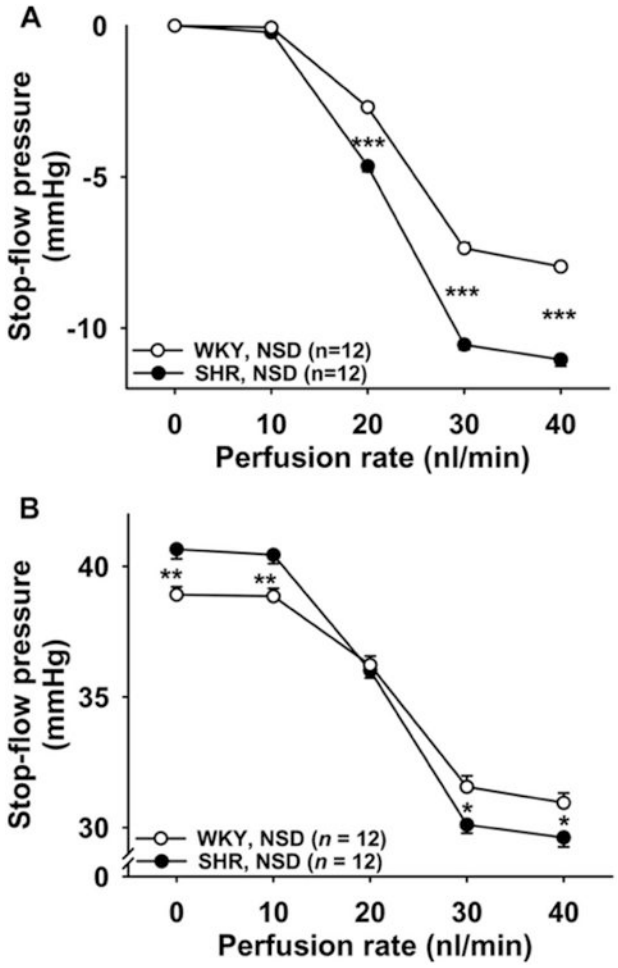

Figure 4. TGF response in WKY and SHR fed normal salt diet

TGF induced by increased perfusion rates in the late proximal tubule in WKY (○) and SHR (●) on a normal salt diet (NSD). When the tubules were perfused at 20, 30, and 40 nL/min, TGF was significantly enhanced in SHR. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, WKY *vs*. SHR Data are presented as delta from baseline (Panel A) and absolute numbers (Panel B).

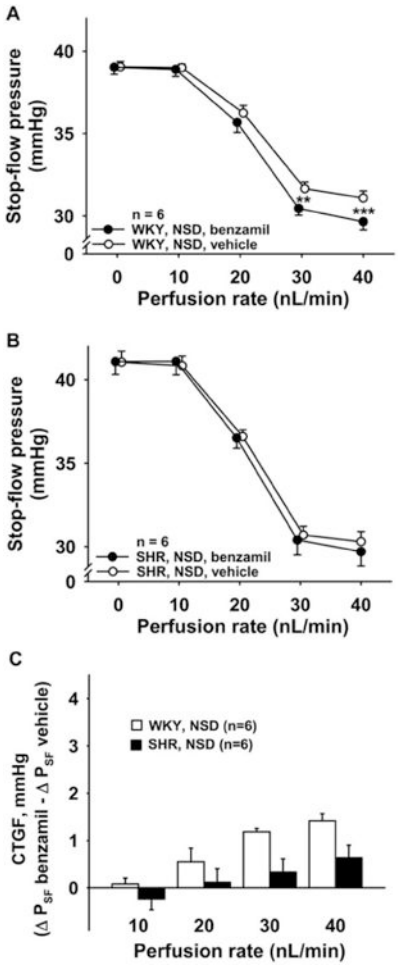

Figure 5. Role of CTGF in TGF response in WKY and SHR fed normal salt diet

In rats fed a normal salt diet (NSD), two TGF responses were elicited, first in the presence of CTGF (○, vehicle), then during inhibition of CTGF (●, benzamil 1μM). Inhibition of CTGF potentiated the TGF response in WKY (Panel A) but not in SHR (Panel B). Panel C: SHR (closed bars) tended to have a smaller CTGF response compared to WKY (open bars). $P < 0.01$ for the overall comparison between strains (ANOVA) and $P < 0.05$ at the 30 and 40 nL/min perfusion rates, but not statistically significant after adjustment for multiple comparisons.

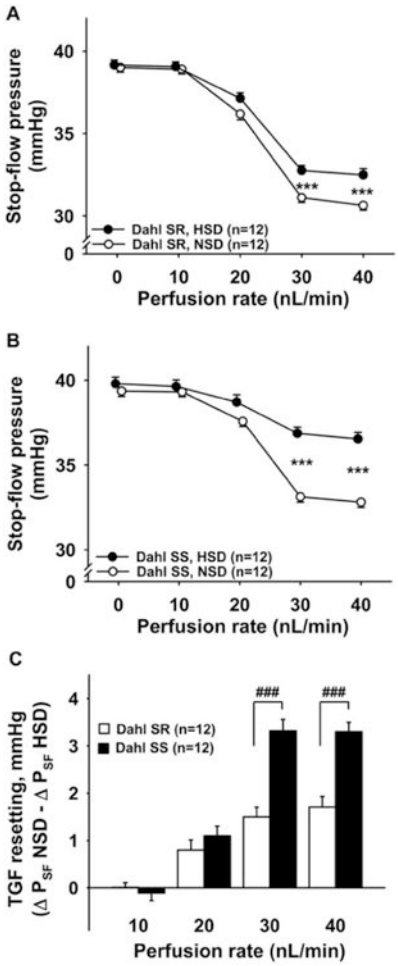

Figure 6. TGF resetting induced by high-salt diet (two weeks) in Dahl SR and Dahl SS

TGF resetting was calculated as the difference in ΔP_{SF} on a normal salt diet (NSD) minus ΔP_{SF} on a high-salt diet (HSD). Panels A and B show that a high-salt diet causes TGF resetting in Dahl SR (Panel A) and Dahl SS (Panel B). Panel C shows the inter-strain comparison of TGF resetting. Dahl SS (closed bars) have significantly higher TGF resetting than Dahl SR (open bars). *** $P < 0.001$, NSD *vs*. HSD; $^{###}P < 0.001$, Dahl SR *vs*. Dahl SS.

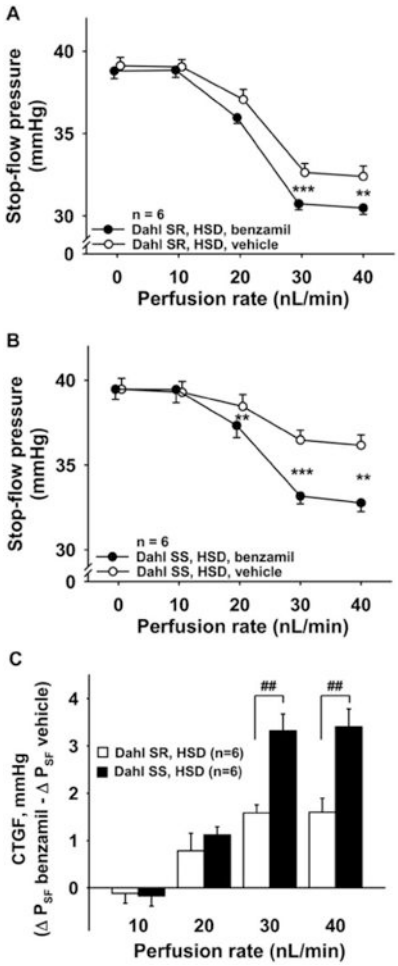

Figure 7. Role of CTGF in TGF response in Dahl SR and Dahl SS fed high-salt diet

In rats fed a high-salt diet (HSD), two TGF responses were elicited, first in the presence of CTGF (○, vehicle), then during inhibition of CTGF (●, benzamil 1μM). Inhibition of CTGF potentiated the TGF response in both Dahl SR (Panel A) and Dahl SS (Panel B). Panel C: CTGF response in Dahl SR (open bars) and SS (closed bars). Dahl SS had greater CTGF responses than Dahl SR when fed a high-salt diet. $^{**}P < 0.01$ $^{***}P < 0.001$, vehicle *vs*. benzamil. $^{\#\#}P < 0.01$, Dahl SR *vs*. Dahl SS.

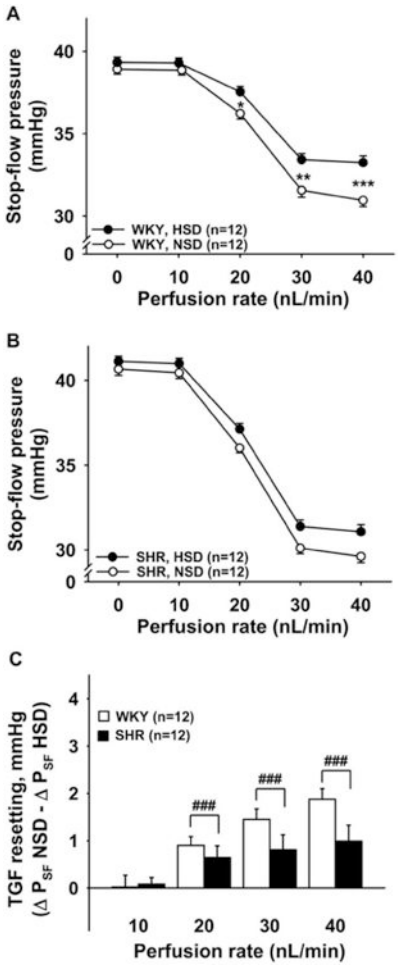

Figure 8. TGF resetting induced by high-salt diet (two weeks) in WKY and SHR

TGF resetting was calculated as the difference in ΔP_{SF} on a normal salt diet (NSD) minus ΔP_{SF} on a high-salt diet (HSD). Panels A and B show that a high-salt diet causes TGF resetting in WKY (Panel A) but no significant TGF resetting in SHR (Panel B). Panel C shows the inter-strain comparison of TGF resetting. SHR (closed bars) have significantly lower TGF resetting than WKY (open bars). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, NSD *vs*. HSD; $^{\#\#\#}P < 0.001$, WKY *vs*. SHR.

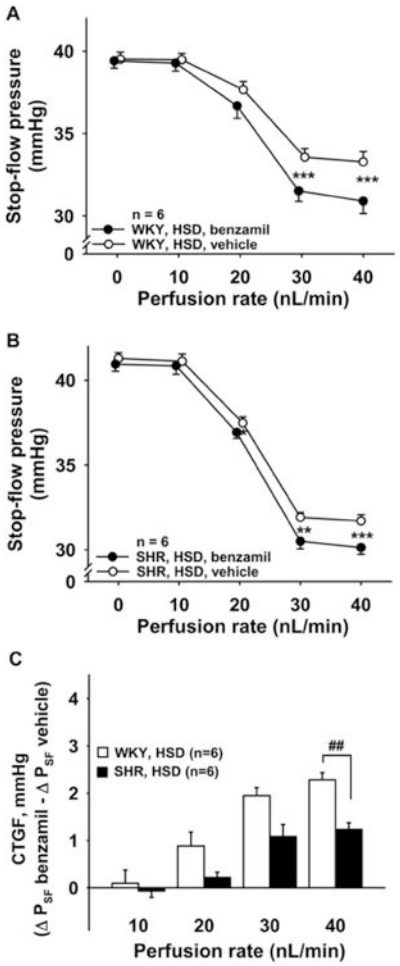

Figure 9. Role of CTGF in TGF response in WKY and SHR fed high-salt diet

In rats fed a high-salt diet (HSD), two TGF responses were elicited, first in the presence of CTGF (○, vehicle), then during inhibition of CTGF (●, benzamil 1μM). Inhibition of CTGF potentiated the TGF response in both WKY (Panel A) and SHR (Panel B). Panel C: CTGF response in WKY (open bars) and SHR (closed bars). SHR had lower CTGF responses than WKY when fed a high-salt diet. $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, vehicle *vs.* benzamil. $^{\#\#\#}P < 0.001$, WKY *vs.* SHR.

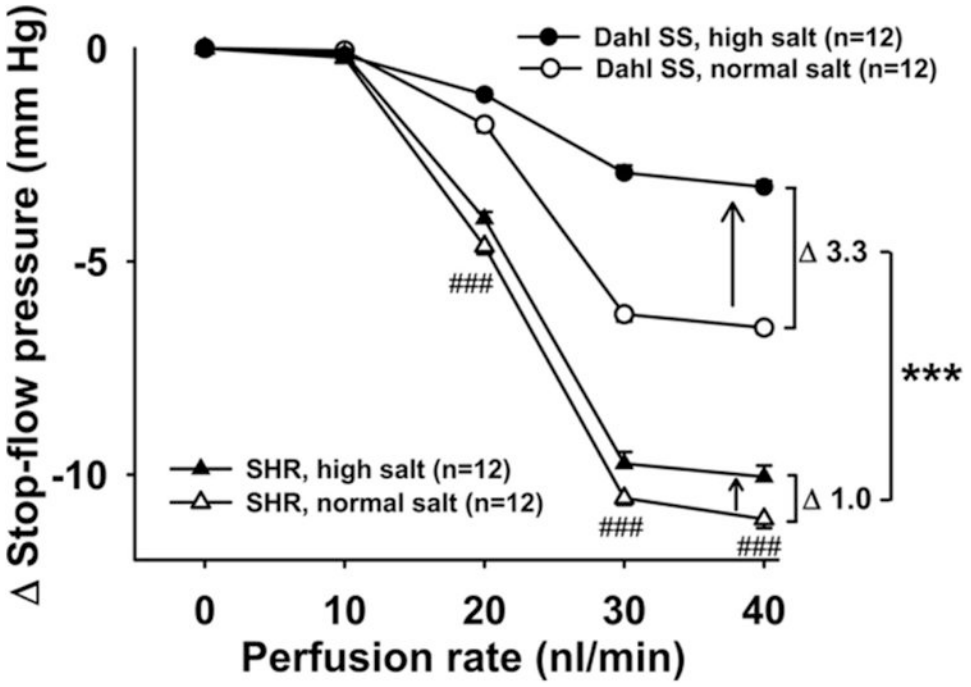

Figure 10. TGF resetting induced by high-salt diet (two weeks) in Dahl SS and SHR

TGF resetting was calculated as the difference in ΔP_{SF} on a normal salt diet (NSD) minus ΔP_{SF} on a high-salt diet (HSD). Dahl SS have an attenuated TGF response compared to SHR, even when fed a normal salt diet. $^{###}P < 0.001$, Dahl SS normal salt *vs*. SHR normal salt. Furthermore, when fed a high-salt diet for two weeks, the difference between strains becomes even greater. This is because high salt attenuates the response in both strains, but to a greater extent in Dahl SS. $^{***}P < 0.001$, TGF resetting in Dahl SS *vs*. TGF resetting in SHR.

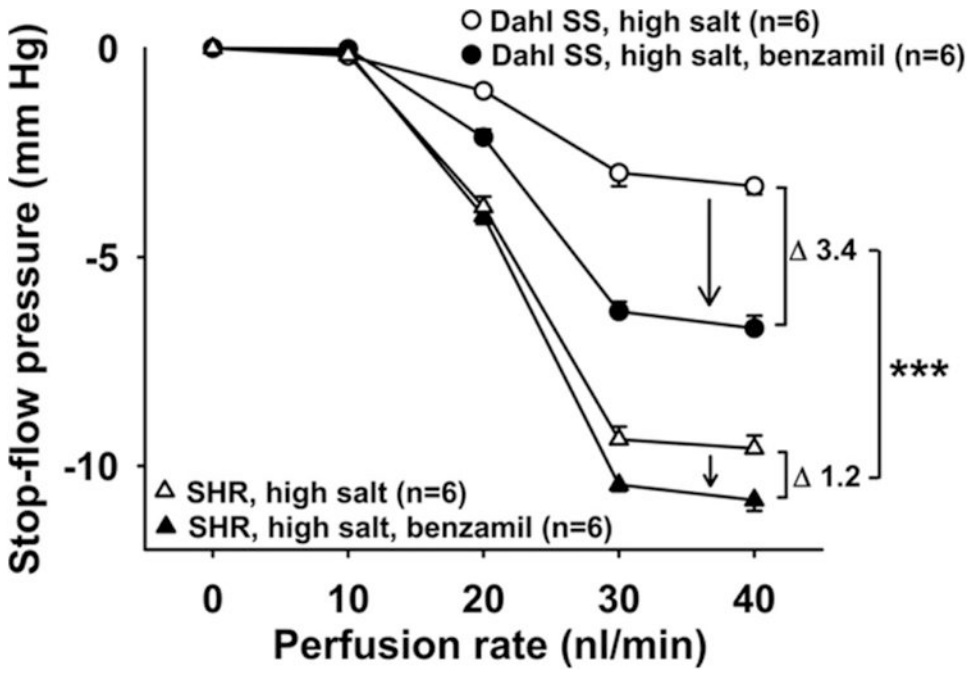

Figure 11. Role of CTGF in TGF response in Dahl SS and SHR fed high-salt diet

In rats fed a high-salt diet (HSD), two TGF responses were elicited, first in the presence of CTGF (○, vehicle), then during inhibition of CTGF (●, benzamil 1μM). Inhibition of CTGF potentiated the TGF response by 13 ± 2% in SHR (1.2 ± 0.1 mm Hg in absolute numbers) and by 83 ± 22 % in Dahl SS (3.4 ± 0.4 mm Hg in absolute numbers). Dahl SS had significantly greater CTGF than SHR when fed high salt. *** $P < 0.001$, CTGF in Dahl SS *vs*. CTGF in SHR.