Resetting of exaggerated tubuloglomerular feedback activity in acutely volume-expanded young SHR

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Brännström, Kristina, and William J. Arendshorst. Resetting of exaggerated tubuloglomerular feedback activity in acutely volume-expanded young SHR. Am. J. Physiol. 276 (Renal Physiol. 45): F409–F416, 1999.—One purpose of the present study was to evaluate the ability of 7-wk-old spontaneously hypertensive rats (SHR) to reset tubuloglomerular feedback (TGF) activity in response to acute volume expansion (VE). Second, we evaluated the contribution of ANG II, via its action on AT1 receptors, to TGF control of glomerular function during VE. TGF was assessed by micropuncture methods and proximal tubular stop-flow pressure (SFP) determinations in SHR, Wistar-Kyoto rats (WKY), and Sprague-Dawley rats (SD). During eu- and hypovolemia SHR exhibited enhanced TGF activity. In the same animals acute VE was achieved by infusion of saline (5 ml·h⁻¹·100 g body wt⁻¹). VE led to resetting of TGF in all three strains. Maximal SFP responses, elicited by a 30–40 nl/min loop of Henle perfusion rate, decreased from 19 to 12 mmHg in SHR and, on average, from 11 to 5 mmHg in WKY and SD (P < 0.001). Tubular flow rate producing a half-maximal response (turning point) shifted to higher flow rates during VE, from 12 to 14 nl/min in SHR and from 15 to 19 nl/min in WKY. Administration of the AT1 receptor blocker candesartan (0.05 mg/kg iv) during sustained VE decreased TGF-mediated reductions in SFP in SHR and slightly increased the turning point in WKY. Nevertheless, other parameters of TGF activity were unaffected by AT1 receptor blockade. In conclusion, young SHR possess the ability to reset TGF activity in response to VE to a degree similar to compensatory adjustments in WKY. However, TGF remains enhanced in SHR during VE. ANG II and its action on AT1 receptors are in part responsible for the exaggerated SFP responses in young SHR during VE.

TGF is a crucial modulator of tubuloglomerular feedback activity. A resetting of TGF in response to acute volume expansion can be reversed by systemic infusion of ANG II (23). Accordingly, resetting of TGF activity associated with changes in hydration state is thought to be due, at least in part, to changes in renin activity and ANG II levels. More recent studies have shown that a resetting of TGF may occur in response to a sustained increase in late proximal flow, in the absence of changes in the systemic nerve activity or levels of circulating hormones (28).

Little is known about the ability of genetically hypertensive rats to reset TGF activity and whether the degree of resetting differs from that of normotensive animals. One study shows that hypertensive Milan rats are resistant to the usual resetting of TGF activity associated with acute volume expansion or unilateral ureteral obstruction. In contrast, the TGF response in normotensive rats is almost abolished in these experimental states (3, 18). The ability of 6- to 7-wk-old SHR to reset TGF activity has been investigated in response to chronic salt loading (32). The investigators found a more pronounced attenuation of TGF activity in WKY compared with SHR, but only when tubular fluid...
collected from salt-loaded animals was used to perfuse Henle’s loop instead of artificial tubular fluid. In contrast to chronic expansion, similar resetting is observed in acutely volume-expanded animals when nephrons are perfused with artificial or native tubular fluid (25). The ability of young SHR to reset TGF activity in response to acute volume expansion has to our knowledge not been investigated.

The first objective of the present study was to ascertain the ability of young SHR to reset TGF activity in response to acute saline volume expansion (5% body wt/h). Second, the role of endogenous ANG II and its action on AT1 receptors were determined during volume expansion to evaluate the contribution of the AT1 receptors to TGF activity during acute saline volume expansion. TGF characteristics were evaluated in young SHR, WKY, and Sprague-Dawley rats (SD) by measuring stop-flow pressure (SFP) during different rates of perfusion of the loop of Henle.

METHODS

Experiments were performed on 7-wk-old SHR, age-matched WKY, and SD obtained from our Chapel Hill breeding colonies using standard methodology for our laboratory. The rats were allowed free access to food and water until the day before the experiment, when food, but not water, was removed. The rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (65 mg/kg body wt) and the body temperature was kept at 37.5°C by placing the animals on a servo-regulated heating table. Tracheotomy was performed to facilitate spontaneous breathing. The left jugular vein was cannulated for supplementary doses of anesthetic and for administration of isoncotic bovine serum albumin (4.7 g/dl). The initial rate of albumin administration was 50 µl/min to replace losses during surgery (1.25 ml/100 g body wt) and then 5 µl/min for the duration of an experiment. Previous studies in our laboratory have demonstrated that this protocol maintains hematocrit and plasma protein concentration at presurgical levels during control conditions, termed euvolemia (10). The right femoral artery was cannulated to obtain blood samples and to record mean arterial pressure (Statham P23Db transducer). The right femoral vein was cannulated for saline infusion during surgery and euvolemic periods at a rate of 10 µl/min. The bladder was cannulated to allow free urine flow. The left kidney was exposed through an abdominal midline incision, dissected free from adhering tissue, and placed in a Lucite cup. The kidney was loosely surrounded with saline-soaked cotton, and the cup was filled with a 3% agar solution. The kidney surface was covered with saline to prevent drying.

After surgery the animals were allowed to stabilize for 1 h before observations were started. TGF characteristics were determined in superficial nephrons by proximal tubular SFP determinations, an index of glomerular capillary pressure, using standard micropuncture methods combined with orthograde perfusion of Henle’s loop as described previously (3, 7). When SFP measurements were performed, random proximal convolutions were punctured with a sharpened glass pipette (OD 3–4 µm) filled with a 2-M NaCl solution stained with Fd&G #3 green dye as control. A third pipette (OD 7–8 µm) was used to inject an immobile wax block in a segment of the mid-proximal tubule to obtain SFP and to isolate the pressure and perfusion pipettes. The SFP was measured in an early proximal convolution while the loop of Henle was perfused at different rates between 0 to 40 nl/min, starting either at 40 or 0 nl/min. The perfusion rate was changed in steps of 2.5–5 nl/min and kept at each rate for 1–5 min, as required to observe a stable response. The maximum decrease in SFP was recorded at high rates of perfusion (30–40 nl/min). The perfusion rate that elicits a half-maximal decrease in SFP, designated as turning point (TP), and slope at the TP or reactivity was calculated by best-fit regression analysis of data for each nephron using a logistic equation and SigmaPlot (Jandel, San Rafael, CA):

\[ SFP = SFP_{min} + \frac{\Delta SFP}{1 + \exp\left(-\frac{4R(\Delta SFP)(PR - TP)}{2}\right)} \]

where PR is the perfusion rate for each recorded pressure level and SFP_{min} is the minimum SFP (3, 7).

Acute saline volume expansion. Measurements of SFP were performed during a control period of euvolemia and during volume expansion. The animals were acutely expanded with isotonic saline after TGF measurements in the control period. Saline was infused intravenously at a rate of 5% of body wt (5 ml·100 g body wt⁻¹·h⁻¹). The animals were allowed to stabilize for 1 h, and the saline infusion rate was adjusted to match urine flow to reach a new steady state before TGF determinations during the experimental period were begun.

Candesartan during acute saline volume expansion. In other animals, acute saline volume expansion was initiated on completion of surgery. Saline was infused intravenously at a rate of 5% of body wt/h (5 ml·100 g body wt⁻¹·h⁻¹). The animals were allowed to stabilize for 1 h, and the saline infusion rate was adjusted to match urine flow to reach a new steady state. Blood samples for hematocrit determinations were taken during surgery, during the volume expansion control period, and at the end of the experiment after candesartan administration. SFP determinations were made during the control period of volume expansion and after intravenous injection of candesartan during sustained volume expansion in an experimental period. The receptor antagonist was given systemically in a bolus dose of 0.05 mg/kg, and measurements were restarted 30 min after the injection. This dose was chosen because it blocked receptors in the renal vasculature without affecting mean arterial blood pressure in pilot studies. All measurements were tested by Student’s t-test for strain difference and intervention effect using SigmaStat software (Jandel). Values are means ± SE. P < 0.05 was considered statistically significant.

RESULTS

Age, left kidney weight, and body mass of WKY and SHR within each protocol were similar (Table 1). The SD were younger and had a larger body and left kidney weight than the other animals. Hematocrit was similar in all three strains within each protocol during euvolemia and volume expansion. Hematocrit fell significantly immediately after initiation of acute saline volume expansion. Thereafter it remained constant during the sustained expansion after candesartan administration. Mean arterial pressure was higher in
7-wk-old SHR compared with the normotensive strains. Mean arterial pressure was quite stable throughout the experiments in all three strains.

To evaluate the completeness of the blockade of AT1 receptors, ANG II (5 ng/10 µl saline; Cheminert sample injection valve) was injected intravenously in each animal during volume expansion before and after candesartan administration. Mean arterial blood pressure and superficial cortical blood flow (laser-Doppler) were monitored continuously. Injection of ANG II during basal conditions caused 10–15% peak increase in mean arterial pressure and 15% maximum decrease in superficial cortical blood flow of the experimental kidney (Fig. 1). Both of these pressor responses were blocked by 95% in each animal after candesartan administration.

Proximal free-flow pressure was 12 mmHg in all strains during the euvolemic control period (Table 2). Acute saline volume expansion caused an increase in tubular free-flow pressure to 15–18 mmHg in the three strains of rats. SFPs were similar in all three strains during euvolemia, averaging 40–42 mmHg in SD, WKY, and SHR. During acute saline volume expansion, however, SFP tended to fall slightly in SHR and increased slightly in WKY and SD. Sigmoidal SFP response curves during euvolemia and acute saline volume expansion for SHR and WKY are shown in Fig. 2. SFP measurements during perfusion of Henle’s loop with artificial tubular fluid at different rates revealed an exaggerated TGF activity in young euvolemic SHR, confirming previous results from this and other laboratories (4, 10, 32). The exaggerated TGF activity was evidenced by a greater maximal SFP response in young SHR (19 vs. 11 mmHg in WKY and SD) and a lower TP (12 in SHR vs. 15–16 nl/min in normotensive rats). Young euvolemic SHR also displayed a greater reactivity, observed as a steeper slope at the TP (−5.6 vs. −2.6 mmHg·nl⁻¹·min⁻¹). Acute saline volume expansion was associated with an attenuation of TGF activity in both SHR and WKY and also in SD. Accordingly, the SFP response curves were shifted up and to the right in all groups. Nevertheless, the SFP response curve for acutely volume-expanded SHR remained stronger and positioned to the left compared with the response curves of volume-expanded normotensive rats. Thus the average maximal SFP response was greater in volume-expanded SHR compared with that in the normotensive rats (12 vs. 5 mmHg) and TP was lower (14 vs. 18–19 nl/min). Volume expansion reduced reactivity in all three groups. However, reactivity re-

### Table 1. Age, weights, arterial pressure, and hematocrit in SD, WKY, and SHR during euvolemic control and volume-expansion periods and during volume-expansion and candesartan periods

<table>
<thead>
<tr>
<th></th>
<th>Body Wt, g</th>
<th>Age, days</th>
<th>Left Kidney Wt, g</th>
<th>Hctcontrol, %</th>
<th>Hctexp, %</th>
<th>MAPcontrol, mmHg</th>
<th>MAPexp, mmHg</th>
<th>No. of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Euvolemia/volume expansion</strong></td>
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<td></td>
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<tr>
<td>SD</td>
<td>194 ± 11</td>
<td>44 ± 1</td>
<td>1.16 ± 0.10</td>
<td>50 ± 2</td>
<td>45 ± 3*</td>
<td>112 ± 3</td>
<td>112 ± 2</td>
<td>4</td>
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<tr>
<td>WKY</td>
<td>158 ± 11‡</td>
<td>50 ± 1‡</td>
<td>0.82 ± 0.03‡</td>
<td>49 ± 1</td>
<td>45 ± 1*</td>
<td>120 ± 2</td>
<td>116 ± 3</td>
<td>6</td>
</tr>
<tr>
<td>SHR</td>
<td>138 ± 6‡</td>
<td>50 ± 1‡</td>
<td>0.82 ± 0.02‡</td>
<td>47 ± 1</td>
<td>43 ± 1*</td>
<td>142 ± 2‡</td>
<td>144 ± 3‡</td>
<td>6</td>
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</tr>
<tr>
<td>WKY</td>
<td>156 ± 7</td>
<td>49 ± 1</td>
<td>0.76 ± 0.03</td>
<td>46 ± 1</td>
<td>46 ± 1</td>
<td>122 ± 3</td>
<td>117 ± 4</td>
<td>5</td>
</tr>
<tr>
<td>SHR</td>
<td>147 ± 6</td>
<td>48 ± 1</td>
<td>0.75 ± 0.02</td>
<td>45 ± 1</td>
<td>45 ± 1</td>
<td>139 ± 2†</td>
<td>133 ± 2†</td>
<td>6</td>
</tr>
</tbody>
</table>

All values are means ± SE. Hctcontrol, hematocrit during control period; Hctexp, Hct during experimental period; MAPcontrol, mean arterial pressure during control period; MAPexp, MAP during experimental period; SD, Sprague-Dawley; WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rats. * P < 0.05 vs. control period; † P < 0.05 vs. WKY; ‡ P < 0.05 vs. SD.

Fig. 1. Renal cortical blood flow (A) and mean arterial pressure (B) responses in 7-wk-old Wistar-Kyoto rats (WKY; n = 5) and spontaneously hypertensive rats (SHR; n = 6) during control conditions of acute volume expansion and after AT1 receptor blockade with candesartan during sustained volume expansion. Values are means ± SE.
mained greater in SHR (−1.5 vs. −0.8 mmHg·nl⁻¹·min⁻¹ WKY and −1.3 mmHg·nl⁻¹·min⁻¹ in SD).

In the second series of experiments, free-flow proximal tubular pressures were similar between SHR and WKY during the control period of acute volume expansion. They remained unchanged during candesartan treatment and sustained volume expansion (Table 2). Proximal SFPs in unperfused nephrons were similar in SHR and WKY (42 vs. 45 mmHg) during the control period. During blockade of AT₁ receptors SFP was slightly lower in SHR (40 vs. 44 mmHg in WKY). Figure 3 shows SFP response curves for SHR and WKY during acute volume expansion before and after candesartan treatment. Blockade of ANG II actions mediated via AT₁ receptors during a volume-expanded state caused a shift in the TP in WKY, from 17 to 21 nl/min. Candesartan had no effect on the maximal change in SFP (6.2 vs. 5.5 mmHg during AT₁ receptor blockade) or reactivity.

Table 2. Summary of paired TGF characteristics in 7-wk-old WKY, SHR, and SD during euvo lemia and volume expansion and before and after candesartan treatment during volume expansion

<table>
<thead>
<tr>
<th></th>
<th>Free-Flow Pressure, mmHg</th>
<th>Stop-Flow Pressure, mmHg</th>
<th>Max ΔSFP, mmHg</th>
<th>Turning Point, nl/min</th>
<th>Reactivity, mmHg·nl⁻¹·min⁻¹</th>
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</thead>
<tbody>
<tr>
<td><strong>SD</strong></td>
<td></td>
<td></td>
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<tr>
<td>Euvolemia</td>
<td>12.3 ± 0.7</td>
<td>42.0 ± 2.0</td>
<td>10.6 ± 0.4</td>
<td>16.3 ± 0.5</td>
<td>−2.6 ± 0.5</td>
<td>7/4</td>
</tr>
<tr>
<td>Volume expansion</td>
<td>18.5 ± 0.4*</td>
<td>43.7 ± 1.3</td>
<td>4.9 ± 0.4*</td>
<td>18.3 ± 0.4*</td>
<td>−1.3 ± 0.2*</td>
<td>9/4</td>
</tr>
<tr>
<td><strong>WKY</strong></td>
<td></td>
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<tr>
<td>Euvolemia</td>
<td>12.2 ± 0.3</td>
<td>40.1 ± 1.1</td>
<td>11.0 ± 0.8</td>
<td>15.3 ± 0.4</td>
<td>−2.6 ± 0.4</td>
<td>12/6</td>
</tr>
<tr>
<td>Volume expansion</td>
<td>16.3 ± 0.5*</td>
<td>42.7 ± 1.1</td>
<td>5.3 ± 0.4*</td>
<td>18.9 ± 0.4*</td>
<td>−0.8 ± 0.1*</td>
<td>10/6</td>
</tr>
<tr>
<td><strong>SHR</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Euvolemia</td>
<td>12.0 ± 0.3</td>
<td>39.6 ± 1.9</td>
<td>18.6 ± 0.8†‡</td>
<td>11.8 ± 0.5†‡</td>
<td>−5.6 ± 0.8†‡</td>
<td>9/6</td>
</tr>
<tr>
<td>Volume expansion</td>
<td>15.4 ± 0.8†‡</td>
<td>38.4 ± 1.4†‡</td>
<td>11.9 ± 0.7†‡</td>
<td>14.1 ± 0.8†‡</td>
<td>−1.5 ± 0.2†‡</td>
<td>10/6</td>
</tr>
<tr>
<td><strong>WKY</strong></td>
<td></td>
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<tr>
<td>Volume expansion</td>
<td>17.2 ± 0.5</td>
<td>44.7 ± 1.2</td>
<td>6.2 ± 0.5</td>
<td>17.3 ± 0.4</td>
<td>−1.0 ± 0.2</td>
<td>9/5</td>
</tr>
<tr>
<td>Volume expansion and candesartan</td>
<td>16.7 ± 0.5</td>
<td>44.5 ± 1.0</td>
<td>5.5 ± 0.4</td>
<td>21.4 ± 0.5*</td>
<td>−1.0 ± 0.5</td>
<td>10/5</td>
</tr>
<tr>
<td><strong>SHR</strong></td>
<td></td>
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</tr>
<tr>
<td>Volume expansion</td>
<td>17.5 ± 0.6</td>
<td>42.1 ± 1.3</td>
<td>11.0 ± 0.9†</td>
<td>15.6 ± 1.0</td>
<td>−1.5 ± 0.3</td>
<td>11/6</td>
</tr>
<tr>
<td>Volume expansion and candesartan</td>
<td>16.9 ± 0.3</td>
<td>39.5 ± 1.2†</td>
<td>7.9 ± 0.7†</td>
<td>16.4 ± 0.5†</td>
<td>−1.2 ± 0.2</td>
<td>15/6</td>
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Values are means ± SE. TGF, tubuloglomerular feedback; ΔSFP, stop-flow pressure response; N/A, nos. of nephrons and animals. *P < 0.05 control vs. experimental period; †P < 0.05 vs. WKY; ‡P < 0.05 vs. SD within a protocol.

Fig. 2. Changes in stop-flow pressure in 7-wk-old WKY (A; n = 6) and SHR (B; n = 6) produced by different rates of loop of Henle perfusion during euvo lemia and during acute volume expansion. Values are means ± SE.
In contrast, maximum SFP response in SHR to supranormal perfusion rates was decreased from 11 to 8 mmHg while reactivity remained similar (-1.2 vs. -1.5 mmHg·nl⁻¹·min⁻¹ during AT₁ receptor blockade). Thus blockade of ANG II actions through AT₁ receptors produced an upward shift of the SFP response curve in SHR and a slight rightward shift in WKY.

**DISCUSSION**

One goal of the present study was to examine resetting of TGF activity in response to acute saline volume expansion in 7-wk-old SHR compared with changes in WKY and SD. We found that young SHR respond appropriately to acute volume expansion. Resetting of TGF in SHR was evidenced by a decrease in maximum SFP response and reactivity and an increase in TP. In agreement with earlier studies, we observed resetting with roughly 50% attenuation of maximum TGF responses in the two strains of normotensive rats (1). Acute saline volume expansion caused similar absolute changes in maximum SFP responses in SHR and the normotensive controls. It is noteworthy that TGF was exaggerated in young SHR compared with normotensive rats during euvoemlia and that the enhanced activity persisted during acute volume expansion. We previously showed that the augmented TGF responses in 7-wk-old euvoemlia SHR are primarily due to ANG II and can be normalized by blockade of AT₁ receptors (4).

The phenomenon of resetting of TGF is important during increased extracellular fluid volume because attenuation of TGF responses allows a greater fluid delivery past the macula densa before a TGF-induced adjustment of nephron GFR occurs. This adjustment in TGF activity contributes to increased natriuresis and diuresis during expansion of extracellular fluid volume and thus facilitates return of blood volume to the initial euvoemlia setpoint. In 7-wk-old SHR, acute saline volume expansion to 3% body wt/h causes a smaller increase in urine flow rate and sodium excretion than in WKY. This subtle strain difference is less readily discernable during volume expansion to 6% body wt/h (C. T. Stier and W. J. Arendshorst, unpublished observations). Furthermore, others have noted that the increase in plasma volume observed in response to chronic

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**Fig. 3. Loop of Henle perfusion-induced changes in stop-flow pressure in WKY (A; n= 5) and SHR (B; n= 6) during a control period of acute volume expansion and during AT₁ receptor blockade by candesartan during sustained volume expansion. Values are means ± SE.**
salt loading was abolished after 4 wk in WKY, whereas SHR at this time point maintained a greater plasma volume than SHR fed a normal salt diet (32).

Enhanced TGF activity may be a common pathogenic feature shared by several strains of genetically hypertensive rats. In addition to SHR it has been observed in the Milan genetically hypertensive strain (3). The mechanism(s) responsible for the exaggerated TGF control of glomerular function appears to differ between these two hypertensive strains, however. TGF activity in 5- to 7-wk-old hypertensive Milan rats seems to be fixed in an overactive mode and is unresponsive to physiological stimuli, such as acute volume expansion. This contrasts with the pronounced resetting observed previously in normotensive rats and in the present study (3). Thus TGF is not fixed in young SHR and is attenuated in an appropriate manner during acute volume expansion. Older hypertensive Milan rats do not reset in response to ureteral occlusion or nitric oxide synthesis inhibition, both of which are very strong stimuli for attenuation of TGF in normotensive animals (18, 30).

Young SHR are able to reset TGF activity in response to a variety of conditions. In two recent studies we have shown attenuation of TGF activity in young euvolemic SHR. AT₁ receptor blockade achieved either by systemic administration of candesartan or intrarenal perfusion with losartan results in TGF resetting in 7-wk-old SHR (4, 5). Resetting in response to chronic salt loading in 6- to 7-wk-old SHR and WKY has been assessed (32). In these microperfusion studies there was more substantial resetting of TGF control of glomerular activity in WKY than in SHR. Interestingly, changes in TGF activity were noted only when native proximal tubular fluid collected from salt-loaded animals was used for orthograde perfusion instead of artificial tubular fluid. Thus a tubular factor appears to be required to modulate function of the juxtaglomerular apparatus during certain conditions, with less effective production or action in young SHR subjected to chronic salt loading. Our data show that TGF resetting occurs using standard methodology and perfusion of Henle’s loop with artificial tubular fluid in young, acutely volume-expanded SHR, WKY, and SD. Moreover, previous studies have failed to detect any differences in TGF responses in nephrons perfused with artificial or native proximal tubular fluid during euvoelma, acute volume expansion, salt restriction, or restricted food intake (21, 25).

Several other different mechanisms have been proposed to be involved in resetting of TGF activity during changes in extracellular fluid volume. For example, modified TGF responses have been reported to be associated with changes in net interstitial pressure, tubular flow rate, and renin-angiotensin activity (19, 23, 28). A recent study suggests that TGF resetting in response to volume expansion may be related to sustained activation of the TGF mechanism per se (28). Increases in late proximal flow rate produced by micropuncture initially saturated TGF, and additional changes in tubular flow rate had no effect. However, the ability of the TGF mechanism to compensate for a flow perturbation recovered over the following 30 min. Whether a sustained increase in tubular flow affects TGF signal sensing at the macula densa, transmission of a signal, or the effector mechanism is unclear.

There is substantial evidence that endogenous ANG II is an important modulator of TGF activity (4, 14, 15, 26). Systemic blockade of angiotensin-converting enzyme in hydropenic rats markedly attenuates TGF responses (14). The modulating effect of ANG II has also been noted using selective ANG II receptor antagonists, avoiding interference with the kallikrein-kinin system. Accordingly, early micropuncture studies in hydropenic rats showed that systemic administration of the nonspecific ANG II receptor antagonist saralasin reduced the maximal TGF response by ~50% (20). Furthermore, infusion of ANG I into the peritubular capillaries enhances TGF activity, an effect that could be blocked by saralasin (15).

Acute volume expansion reduces plasma renin activity, and TGF usually varies in direct proportion to activity of the renin-angiotensin system (23). Furthermore, a change in the renin-angiotensin activity has been proposed as a mechanism for TGF resetting during volume expansion. When renal arterial pressure is kept constant, systemic infusion of ANG II in acutely saline volume-expanded rats reverses attenuation of TGF responses (23). Infusion of ANG II to hydropenic concentrations in volume-expanded rats restores TGF responses to levels observed in hydropenic rats (23). These results suggest that resetting of TGF activity in acutely volume-expanded rats can be explained, at least in part, by a decrease in ANG II levels. Possible differences in roles of systemically versus intrarenally generated ANG II are unclear at present.

More recently we have shown that intrarenal infusion of the specific AT₁ receptor antagonist losartan attenuates TGF activity in euvolemic SHR but not in WKY (4). TGF control of glomerular function was sustained and similar in the two strains during AT₁ receptor blockade. Thus, in our hands, AT₁ receptor blockade does not attenuate TGF responses in either WKY or Munich-Wistar rats during euvoelma when activity of the renin-angiotensin system is low (4). In agreement with our earlier study, our current data indicate that some TGF activity persists during AT₁ receptor blockade. It appears that all ANG II receptors are completely blocked by the pharmacological agent in the present study. We found that the cortical renal blood flow and arterial blood pressure responses to ANG II were almost absent after systemic administration of candesartan. Furthermore, a recent study has shown that a five times lower dose of candesartan results in a marked block of arterial pressure and cortical renal blood flow responses to ANG II, suggesting that at least renal AT₁ receptors are, for practical purposes, completely inhibited (6).

In contrast, an absence of TGF responses has recently been reported in AT₁A receptor-deficient mice,
suggested an obligatory chronic role for ANG II (26). Interestingly, ANG II is able to elicit renal and systemic vasoconstriction responses in AT1A receptor-deficient mice, albeit smaller than in wild-type animals (17). These studies indicate that ANG II action on the TGF mechanism can be dissociated from a renal vasoconstrictor effect in mice devoid of AT1A receptors. Although the exaggerated TGF activity in young SHR during volume expansion was attenuated by candesartan treatment, the present data show that young volume-expanded, AT1 receptor-blocked SHR continue to exhibit enhanced TGF responses. This suggests that factors other than ANG II are involved. During control conditions of euhydration, AT1 receptor blockade with either losartan or candesartan normalizes TGF activity in young SHR, returning maximal SFP responses, TP, and reactivity to WKY values. These results indicate that ANG II antagonists have access to the AT1 receptors of importance for TGF modulation (4, 5).

In addition to ANG II, other factors modulate TGF activity. Examples include adenosine, nitric oxide, and thromboxane A2. Also, adenosine may interact with ANG II in a synergistic manner on vascular reactivity (33). Recent studies have shown enhancement of TGF responses during nitric oxide synthesis blockade in normotensive rats but not in hypertensive Milan rats or SHR (30, 31). Nitric oxide can counteract the vasoconstrictor action of ANG II in a manner similar to the buffering of ANG II-induced vasoconstriction by prostaglandins. Intrarenal administration of ANG II produces exaggerated renal vasoconstriction in young SHR compared with WKY, indicating an increased vascular sensitivity to ANG II in SHR (8, 9). This increased reactivity is probably due to blunted counteracting effects of cyclooxygenase products (8). A modulating factor of TGF that has been reported to act independently of ANG II is thromboxane A2 (34). In normotensive rats administration of a thromboxane A2 antagonist results in an attenuated TGF response and when given together with the angiotensin antagonist saralasin causes an additive blunting. The influence of thromboxane A2 on TGF in SHR is not known.

In summary, our results demonstrate that young SHR possess the ability to reset TGF regulation of glomerular function in response to acute saline volume expansion. Attenuated TGF activity is evidenced by a decrease in maximal SFP response and reactivity and an increase in TP. However, an enhanced TGF activity persists in volume-expanded young SHR compared with WKY. AT1 receptor blockade reduces the maximum SFP responses in SHR during sustained acute volume expansion. Nevertheless, TGF activity remains enhanced in SHR after candesartan administration. These findings suggest that ANG II and its action via AT1 receptors play a role in the exaggerated TGF control of glomerular function in acutely volume-expanded young SHR.

Candesartan was kindly provided by Astra Hässle, Sweden. This study was supported by Research Grant HL-02334 from the National Heart, Lung, and Blood Institute. Kristina Brännström was supported by a predoctoral fellowship from the American Heart Association, NC.

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Received 10 August 1998; accepted in final form 15 October 1998.