Vascular endothelial growth factor administration does not improve microvascular disease in the salt-dependent phase of post-angiotensin II hypertension

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Submitted 22 March 2006; accepted in final form 22 June 2006

...-dependent phase of post-angiotensin II hypertension. Therefore, we hypothesized that vascular endothelial growth factor (VEGF) administration would prevent the development of salt-sensitive hypertension induced by ANG II. Infusion of ANG II in rats for 2 wk led to an elevation in blood pressure and an increase in blood urea nitrogen. Prominent tubular injury, focal areas of peritubular capillary loss accompanied by a decrease in urinary nitrates, thickening of the afferent arteriole, and an elevation in systemic and renal VEGF protein levels also occurred. In separate studies, animals were infused with ANG II and then placed on a low-salt diet for 1 wk. At this point, the animals were paired on the basis of weight and blood pressure and treated with either VEGF121, or vehicle subcutaneously for 8 wk while being fed a high-salt diet. During the treatment period, a spontaneous improvement in many parameters, including both renal function and healing of the peritubular capillaries, occurred to the same degree in both vehicle- and VEGF121-treated rats. VEGF121 significantly reduced blood pressure and accelerated the recovery of tubular injury. In contrast, vehicle-treated rats demonstrated a persistent increase in afferent arteriolar media-to-lumen ratio, which was further enhanced in rats treated with VEGF121. Therefore, VEGF therapy has only limited benefits on the healing of renal lesions in the salt-dependent phase of post-ANG II-mediated hypertension.

blood vessels; vascular growth factors

SYSTEMIC HYPERTENSION AFFECTS 20–30% of the population in the United States and other developed countries (13). Epidemiological and interventional studies have strongly linked salt intake with hypertension. Complex alterations in the kidney contribute to salt-sensitive hypertension. Recent evidence has indicated that microvascular injury and tubulointerstitial inflammation may provide one mechanism for the development of salt-sensitive hypertension (12). Ischemia resulting from arteriolar disease and peritubular capillary loss results in the recruitment of inflammatory cells with the local generation of oxidants and vasoconstrictive substances that perpetuate vasoconstriction and sodium retention (32).

The above pathway suggests that interventions aimed at improving the microvasculature might provide a means for preventing or treating salt-sensitive hypertension. One way this potentially could be achieved is through the administration of growth factors that mediate vascular differentiation and integrity (34). Vascular endothelial growth factor (VEGF) stimulates endothelial cell proliferation and migration, blocks endothelial cell apoptosis, and stimulates new capillary growth (34). VEGF mRNA and protein are expressed in glomeruli and tubules maintaining renal endothelial cell structure and modulating permeability (6, 27, 29, 41). There is a loss of VEGF in renal diseases including glomerulonephritis (42), acute renal failure (41), and chronic renal disease (15). Administration of VEGF can also ameliorate renal injury in glomerulonephritis induced by Thy-1 (24), anti-glomerular basement membrane antibodies (37), and subtotal renal ablation (14).

Salt-sensitive hypertension can be induced in animals by the administration of agents that initiate transient episodes of renal vasoconstriction. Infusion of either ANG II or cyclosporine results in tubulointerstitial injury, arteriolar lesions, fibrosis, and peritubular capillary loss (10, 17, 20, 21); once injury is induced, the animals become hypertensive after sodium loading. Kang et al. (17) reported that cyclosporine causes a loss of VEGF expression, and the ensuing salt-sensitive hypertension is attenuated by administration of VEGF121 (one of several isoforms of VEGF). The lowering of blood pressure was associated with a reduction in tubulointerstitial damage and a decrease in afferent arteriolar hyalinosis (arteriolopathy) (17). Given these findings, we hypothesized that VEGF121 administration would also prevent the development of salt-sensitive hypertension induced by ANG II.

MATERIALS AND METHODS

Animals and reagents. Adult male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing 150–180 g were housed in a temperature- and light-controlled environment. Animal procedures were approved by the Animal Care Committee of the University of Florida, Gainesville. All reagents were obtained from Sigma (St. Louis, MO) unless otherwise stated.

Experimental protocol. Experimental strategy and groups are summarized in Figs. 1 and 2, respectively. ANG II (435 ng·kg⁻¹·min⁻¹)...
dissolved in Ringer lactate (Fisher Scientific, Hampton, NY) was administered for 2 wk to rats through subcutaneous osmotic minipumps (Alza, Mountain View, CA) \((n = 23)\) \((10, 20)\). Sham controls received Ringer lactate infusion only \((n = 7)\). During this initial phase of the experiment, all animals were fed a standard laboratory diet and water ad libitum. At the end of 14 days, body weight and blood pressure (BP) were measured. All of the sham control animals and seven rats administered ANG II were placed in individual metabolic cages for 24-h urine collection and then euthanized under anesthesia \((Groups I \text{ and II})\). To obtain accurate nitrite and nitrate measurements, the animals were fasted, beginning 8 h before and extending during the collection period \((1)\). At death, serum, plasma, and kidney tissues were obtained. Minipumps were removed from the remaining animals infused with ANG II, and these rats were placed on a low-salt diet \((0.1\% \text{ NaCl; Harlan, Indianapolis, IN})\) for a 7-day equilibration period. Animals were then paired according to body weight and BP and randomly assigned to vehicle \((n = 7)\) or ANG II \((n = 8, Group III)\) or VEGF121 \((n = 8, Group IV)\) treatment. The diet was then switched to high salt \((4\% \text{ NaCl; Harlan})\) to generate salt-sensitive hypertension, and VEGF121 \((50 \mu g/kg \text{ twice daily subcutaneously})\) or vehicle \((PBS, pH 7.2)\) was administered for 8 wk. To determine whether the effects of VEGF were maintained when the treatment was stopped, VEGF administration was discontinued, with the rats being maintained on a high-salt diet for 5 days before death.

At this dose, VEGF121 attains a peak plasma level of 50 ng/ml at 100 min after injection and remains detectable in the plasma at 5 ng/ml after 6 h \((38)\). These plasma levels of VEGF121 do not affect systemic BP in normal rats \((Terjung R, Abraham J, Schreiner GF, personal communication)\). Urine and serum were collected after 4 wk of treatment and at death as outlined above. To determine whether the effects of VEGF121 were maintained when the treatment was stopped, VEGF121 administration was discontinued, with the rats being maintained on a high-salt diet for 5 days before being euthanized.

**Measurement of BP.** Systolic BP was measured each week in conscious animals between 9 AM and 12 PM by tail-cuff plethysmography \((Visitech Systems, Apex, NC)\). Rats were conditioned at least twice on separate days before the first measurements of BP. Systolic BP values were derived from the median of 5–10 measurements for each animal at each time point.

**Measurement of blood urea nitrogen.** Blood urea nitrogen \((BUN)\) was measured following ANG II infusion, 4 wk after VEGF121 treatment, and at the end of the experiment by a two-step procedure as outlined by the manufacturer \((Diagnostic Chemicals, Oxford, CT)\).

**Immunohistochemistry.** Kidneys were fixed in methyl Carnoy’s solution, and 5-μm sections were dewaxed and rehydrated; some were microwaved with 1.9 g/l citric acid \((pH 6.0)\) to enhance antigen retrieval depending on the antibody used. Endogenous peroxidase was quenched with 3% \(\text{H}_2\text{O}_2\) in methanol for 30 min, and sections were blocked with 10% fetal calf serum, 0.2% BSA, and 0.1% Tween-20 in PBS \((pH 7.4)\). Sections were reacted overnight with monoclonal and polyclonal antibodies: mouse monoclonal antibody JG-12 \((a gift from Dr. Dontschko Kerjaschki, University of Vienna, Austria)\) and rat endothelial cell antigen-1 \((RECA-1; Serotec, Indianapolis, IN)\) directed against rat capillary endothelial cells; polyclonal antibody 199 \((a gift from Dr. Cecillia Giachelli, University of Washington, Seattle, WA)\) to osteopontin \((OPN)\) and mouse monoclonal antibody to α-smooth muscle actin \((α-SMA)\). Bound primary antibodies were detected with appropriate secondary antibodies \((DAKO, Carpinteria, CA)\). Brown color was generated by use of a diaminobenzidine substrate. Negative controls consisted of omission of the primary antibody or replacement of the primary antibody with preimmune serum.

**Quantification of morphological data.** Assessment of immunostaining was performed by an operator blinded to the nature of the specimens. The peritubular capillary rarefaction index \((17)\) was ob-
tained by averaging the percentage of squares in 10 × 10 grids showing no capillaries (by JG-12 and RECA-1 staining) by using at least 10 sequential fields at ×100 magnification. The minimum possible capillary rarefaction index is 0, and the maximum score is 100; the latter would indicate a complete absence of JG-12- and RECA-1-positive cells. The degree of OPN expression in the cortex adjacent to glomeruli in the outer cortex that showed positive α-SMA signals were selected. Afferent arterioles were distinguished from efferent arterioles by the presence of an internal elastic lamina and by thin, flattened endothelial cells (5). Afferent arteriolar wall thickness was measured by computer image analysis using a method previously described (17, 33). For each arteriole, the outline of the vessel and its internal lumen (excluding the endothelium) were generated to calculate the total medial area (outline-lumen) in a minimum of eight arterioles per biopsy. Vessels that were not sectioned transversally, providing an asymmetrical wall, were excluded from the present study. All samples were treated with the same method, and the media-to-lumen ratio was calculated by the outline-lumen relationship.

Western blotting. Kidney cortex tissue was homogenized in cell lysis buffer (Cell Signaling Technology, Beverly, MA) and then centrifuged at 13,000 g for 30 min. The supernatant was removed, and the protein content was measured with a commercially available kit (Bio-Rad Laboratories, Hercules, CA). Eighty micrograms were denatured at 100°C for 5 min and separated on 12% polyacrylamide electrophoresis gels. Proteins were transferred to nitrocellulose membranes (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) by electroblotting (Bio-Rad Laboratories). Blots were blocked for 1 h with 5% (wt/vol) fat-free milk powder, 0.1% BSA, and 0.1% Tween-20 in PBS and incubated at 4°C overnight with rabbit anti-human VEGF polyclonal antibody (1:250, Santa Cruz Biochemicals, Santa Cruz, CA). Blots were washed in PBS with 0.2% Tween-20 and once in blocking solution. They were incubated for 30 min with second antibodies, and bands were detected by chemiluminescence (Amersham Pharmacia Biotech). Proteins were sized with Rainbow markers (Amersham Pharmacia Biotech). As positive controls, rat recombinant VEGF was used (data not shown). Blots were stripped and then reprobed for GAPDH (1:2,500; Abcam, Cambridge, MA) as a housekeeping gene. The intensities of these bands were measured by densitometry and analyzed as the VEGF-to-GAPDH ratio.

Urinary nitrite and nitrate. The final products of nitric oxide (nitrite and nitrate) were measured in the urine by a two-step process (Cayman Chemical, Ann Arbor, MI) (17). The first step was the conversion of nitrate to nitrite utilizing nitrate reductase. The second step was the addition of Greiss reagent that converts nitrite into a deep purple azo compound that can be measured by spectrophotometry.

VEGF and soluble VEGF receptor-1 (sFlt1) ELISA. VEGF serum and kidney cortical sFlt1 levels were measured using a commercially available mouse immunoassay kit (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. These kits previously have been shown to cross-react with rat samples (2, 22).

Statistics. All data were presented as means ± SE unless otherwise stated. Data were analyzed using a one-way ANOVA with Bonferroni post hoc tests. Differences in the various parameters among groups were evaluated by Mann-Whitney U-tests for nonparametric data. A P value <0.05 was considered significant.

RESULTS

Acute effects of ANG II infusion. Consistent with previous reports (10, 20), the infusion of ANG II for 2 wk resulted in the development of hypertension (Fig. 3) with deleterious effects on renal function and histology. ANG II-infused rats showed a significant increase in BUN levels (19.3 ± 0.7 mg/dl in sham control animals vs. 47.3 ± 4.5, P < 0.05) and decreased weight gain (245 ± 9 g in sham control animals vs. 197 ± 13, P < 0.05) at 2 wk.

Kidneys of animals infused with ANG II had focal areas of tubular damage as indicated by an elevated expression of OPN protein levels of sFlt1 in the kidney cortex were similar in both sham and ANG II-infused animals [11.2 ± 2.3 pg/mg protein vs. 13.0 ± 3.0 in sham-operated and ANG II-infused animals, respectively, P = not significant (NS)].

Interestingly, VEGF levels were elevated in ANG II-infused animals. Serum levels of VEGF were modestly elevated in ANG II-infused animals compared with sham infusion (40.6 ± 6.9 pg/ml vs. 55.6 ± 7.8 in sham-operated and ANG II-infused animals, respectively, P < 0.05). VEGF protein levels in the renal cortex as measured by Western blotting were also significantly increased in ANG II-infused rats with a significant decrease in urine nitrite and nitrate levels (1.6 ± 0.7 mM/24 h vs. 0.6 ± 0.3, sham vs. ANG II, P < 0.05). Animals infused with ANG II also had thickened afferent arterioles with a significant elevation in media-to-lumen ratio and total medial area while a reduction in lumen diameter was observed (Table 1, P < 0.05).

Effect of VEGF on ANG II-induced renal injury and salt-sensitive hypertension. In separate studies, rats were administered ANG II for 2 wk followed by removal of the minipumps and placement on a low-salt diet for 1 wk. This allowed the mean systolic BP to fall from 230 mmHg (mean at 14 days) to 148 mmHg. Rats were then paired on the basis of weight and BP, placed on a high-salt diet, and given either vehicle or...
VEGF<sub>121</sub> for 8 wk (n = 8 in each group). At the end of the experimental period, VEGF levels in the kidney cortex (Fig. 6) and in the serum (42.3 ± 6.9 pg/ml vs. 48.0 ± 7.0 in vehicle- and VEGF-treated rats, respectively, P = NS) were similar in both vehicle- and VEGF-treated animals.

VEGF<sub>121</sub>-treated animals were shown to have a significantly lower BP (difference of ~20 mmHg, Fig. 3) than vehicle-treated animals after 6 and 8 wk (P < 0.05). This level of BP was maintained even after the VEGF infusion had been stopped for several days (Fig. 3). However, the difference between the VEGF and vehicle group disappeared because of a spontaneous improvement in BP in the vehicle-treated animals (Fig. 3).

A comparison of VEGF with vehicle was made as it relates to renal function. Interestingly, a spontaneous improvement in vehicle-treated rats was observed, and no differences were noted between VEGF and vehicle-treated rats for many parameters. Thus both renal function, as assessed by BUN levels (21.4 ± 1.4 mg/dl vs. 21.2 ± 1.1 in vehicle- and VEGF-treated animals, respectively, P = NS), and urinary nitrite levels (1.5 ± 0.3 mM/24 h vs. 1.4 ± 0.2 in vehicle- and VEGF-treated animals, respectively, P = NS) returned to levels equivalent to that observed earlier in normal sham controls.

There was also a spontaneous improvement observed in tubular injury (reflected by measurement of OPN expression; Ref. 23) and capillary injury (peritubular capillary rarefaction score) in vehicle-treated rats (Table 1). Although VEGF administration was associated with greater recovery of the tubular injury, as reflected by lower OPN scores compared with vehicle (0.5 ± 0.1% in vehicle-treated animals vs. 0.2 ± 0.1% in VEGF-treated animals, P < 0.05), there was no additional improvement in peritubular capillary density compared with vehicle treatment.

In contrast to the spontaneous recovery of the capillary microvasculature, vehicle-treated rats demonstrated persistent afferent arteriolar thickening, as reflected by both the media-to-lumen ratio and medial wall measurements (Table 1). Importantly, the arteriolar media-to-lumen ratio increased in rats that had been treated with VEGF (Table 1, P < 0.05 vs. vehicle-treated group). This change was due to both a significant decrease in lumen diameter (P < 0.05) and a tendency for medial area to be increased in animals treated with VEGF (Table 1) compared with vehicle-treated animals.

Table 1. Vascular and tubular injury parameters

<table>
<thead>
<tr>
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<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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<tr>
<td>Osteopontin expression, %</td>
<td>0.1±0.05</td>
<td>1.3±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5±0.1&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
<td>0.2±0.05&lt;sup&gt;c,d&lt;/sup&gt;</td>
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<tr>
<td>Peritubular rarefaction index, %</td>
<td>1.4±0.4</td>
<td>10.9±4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7±1.3&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
<td>5.3±1.8&lt;sup&gt;c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Afferent arteriole media/lumen ratio</td>
<td>1.5±0.2</td>
<td>3.4±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.5±0.3&lt;sup&gt;c,d&lt;/sup&gt;</td>
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<tr>
<td>Afferent arteriole luminal diameter, μm</td>
<td>15.4±0.7</td>
<td>13.0±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.9±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.2±0.5&lt;sup&gt;c,d&lt;/sup&gt;</td>
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<tr>
<td>Afferent arteriole medial area, μm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>232.8±28</td>
<td>462±60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>334±24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>368±33&lt;sup&gt;c&lt;/sup&gt;</td>
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Values are means ± SE. *P < 0.05 between Groups I and II. 0.05 < *P < 0.05 between Groups I and III. *P < 0.05 between Groups I and IV. 0.05 < *P < 0.05 between Groups II and III. *P < 0.05 between Groups II and IV. 0.05 < *P < 0.05 between Groups III and IV.
of nitric oxide metabolism; Ref. 1) during the ANG II infusion. A marked reduction in urinary nitrites (which are end products in this study and in the study by Franco et al. (10), we observed relate to the effect of ANG II on renal nitric oxide. Indeed, both 5 wk. Constriction may have subsided in our 8-wk study, and indeed hypertensive response (10). It is possible that the renal vasoconstriction and low glomerular plasma flows are still present 5 wk after stopping the ANG II administration, although we had observed that ANG II can decrease VEGF expression in cultured medullary thick ascending limb tubular epithelial (mTAL) cells (16), it is possible that ANG II-mediated renal vasoconstriction led to renal ischemia that stimulated VEGF expression via hypoxia-inducible factor (HIF)-1α and overrode any direct inhibitory effect on these cell types (30). Interestingly, similar findings occur in patients with scleroderma, a multiorgan disease the features of which include tissue fibrosis, endothelial damage, and fibrosis (11). Scleroderma patients have high serum levels of ANG II (18), and expression of VEGF, VEGF receptor (VEGFR)-1, and VEGFR-2 is upregulated in the skin (4). Additionally, levels of sFlt1, a natural VEGF antagonist (22), were similar following ANG II infusion. Elevated levels of sFlt1 following ANG II could have explained the “resistance to VEGF treatment” in the salt-dependent phase of the model, but our data exclude this possibility. The increase in VEGF observed in our model may have had both beneficial and deleterious effects. Thus, although we have reported that VEGF replacement in situations of local renal deficiency of VEGF can stimulate capillary repair (14, 17), there is also evidence that an overabundance of VEGF may be

Fig. 5. VEGF is elevated systemically and in the kidney cortex following ANG II infusion. Western blotting for VEGF-A in the kidney cortex. Positive signals for VEGF-A were observed at ~50 kDa, the equivalent size of the VEGF dimer. Note the upregulation in ANG II-infused animals. To confirm this, densitometric analysis was performed with all signal intensities compared with levels of the housekeeping gene, GAPDH (all values are means ± SD, P < 0.05 between sham and ANG II-infused animals, n = 6 in each group).

Fig. 6. VEGF protein levels in the kidney cortex following 8 wk of vehicle or VEGF121 treatment. Western blotting for VEGF-A in the kidney cortex. Positive signals for VEGF-A were observed at ~50 kDa, the equivalent size of the VEGF dimer. Densitometric analysis was performed with all signal intensities compared with levels of the housekeeping gene, GAPDH (all values are means ± SD, n = 7 in each group). There was no significant difference between vehicle- and VEGF121-treated animals.

DISCUSSION

In this study we evaluated the potential of an angiogenic factor to improve salt-sensitive hypertension induced by ANG II. The rationale was based on our previous studies suggesting that the BP is driven by microvascular and tubulointerstitial injury induced by ANG II in the kidney and that the amelioration of the renal injury can prevent these hemodynamic changes (10, 32). Because peritubular capillary injury and arteriolar disease develop in this model (20, 35), we hypothesized that the administration of VEGF would stimulate angiogenic repair and reduce ischemic injury, in accordance with our previous demonstrations in other chronic renal diseases (14). However, while VEGF treatment did lead to a reduction in the BP response to a high-salt diet, there also appeared to be a spontaneous improvement in BP in the control group, and, in the end, no difference in either BP or in the renal capillary or tubulointerstitial injury could be shown. While the study as such was negative, there were several interesting findings that were observed.

The first important finding was that there was a spontaneous improvement in BP, renal function, and renal injury in the control rats that had been infused with ANG II and then switched to high-salt diet. We have previously reported that renal vasoconstriction and low glomerular plasma flows are still present 5 wk after stopping the ANG II administration, where they are thought to have a key role in mediating the hypertensive response (10). It is possible that the renal vasoconstriction may have subsided in our 8-wk study, and indeed the spontaneous improvement observed in BP began after 5 wk.

One potential mechanism for the spontaneous repair could relate to the effect of ANG II on renal nitric oxide. Indeed, both in this study and in the study by Franco et al. (10), we observed a marked reduction in urinary nitrites (which are end products of nitric oxide metabolism; Ref. 1) during the ANG II infusion. The urinary nitrites remained depressed for at least 1 wk following the cessation of ANG II but returned to normal levels by 5–8 wk (Ref. 10 and this study). Because nitric oxide is a vasodilator, the increase in nitric oxide could counteract vasoconstriction and thus potentially relieve ischemia.

Another important finding was that acute ANG II infusion resulted in increased plasma and renal VEGF expression. Unlike previous models of renal injury that we have studied, such as the cyclosporine nephropathy model (17) and the remnant kidney model (14), in which VEGF expression in the kidney is decreased, in the ANG II model the opposite was observed. This may be due to the known ability of ANG II to induce VEGF expression in renal smooth muscle, mesangial, and epithelial cells (7, 31, 40). Although we had observed that ANG II can decrease VEGF expression in cultured medullary thick ascending limb tubular epithelial (mTAL) cells (16), it is possible that ANG II-mediated renal vasoconstriction led to renal ischemia that stimulated VEGF expression via hypoxia-inducible factor (HIF)-1α and overrode any direct inhibitory effect on these cell types (30). Interestingly, similar findings occur in patients with scleroderma, a multiorgan disease the features of which include tissue fibrosis, endothelial damage, and fibrosis (11). Scleroderma patients have high serum levels of ANG II (18), and expression of VEGF, VEGF receptor (VEGFR)-1, and VEGFR-2 is upregulated in the skin (4). Additionally, levels of sFlt1, a natural VEGF antagonist (22), were similar following ANG II infusion. Elevated levels of sFlt1 following ANG II could have explained the “resistance to VEGF treatment” in the salt-dependent phase of the model, but our data exclude this possibility.

The increase in VEGF observed in our model may have had both beneficial and deleterious effects. Thus, although we have reported that VEGF replacement in situations of local renal deficiency of VEGF can stimulate capillary repair (14, 17), there is also evidence that an overabundance of VEGF may be
injured, such as in diabetic renal disease (9). Indeed, VEGF is known to stimulate monocyte chemotaxis and vascular smooth muscle cell proliferation via the VEGFR-1 receptor (3, 39). It is thus of interest that afferent arteriolar media-to-lumen ratio increased in rats treated with VEGF. This change was due to both a significant decrease in lumen diameter and a tendency for medial area to be increased. The methods used to calculate these ratios carefully excluded the endothelial layer, so alterations in endothelial cells are not a sufficient explanation for these findings. A recent study has indicated that ANG II infusion in mice increases expression of VEGF in the aortic wall alongside aortic inflammation and remodeling (43) which can be attenuated by the administration of sFlt1. Furthermore, the angiogenic response of VEGF is known to be altered and likely dysfunctional under conditions in which nitric oxide levels are low (26, 28, 36, 44). Indeed VEGF-induced angiogenesis is dysfunctional in the hindlimb ischemia model in mice lacking endotelinal nitric oxide synthase (25). Therefore, we could postulate that, because of reduction in renal nitric oxide following ANG II infusion, VEGF will not improve peritubular capillary rarefaction, particularly at the start of the treatment period. However, it is likely that, as nitric oxide levels recovered, the increased local VEGF might have provided an additional mechanism for the spontaneous recovery of the peritubular capillaries.

One important caveat to consider in this study is whether the VEGF treatment was started too late in the experimental period. Our data indicate that the endothelial injury was most severe at the end of the ANG II infusion. Therefore, it may be that the key contributor to endothelial injury is the initial period of vasoconstriction induced by ANG II and this is the time when VEGF treatment would be most beneficial. However, it should be noted that our experimental design is more likely to represent the clinical situation, as these initial events probably would have already started in patients that require treatment for salt-sensitive hypertension.

GRANTS

Support for this research was provided by National Institutes of Health Grants DK-52121, HL-68607, and DK-64233 (R. J. Johnson); a Bogue Research Fellowship, University College, London (D. A. Long); and a grant from the Kids Kidney Appeal (A. S. Woolf, D. A. Long).

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