AT$_1$ receptor inhibition does not reduce arterial wall hypertrophy or PDGF-A expression in renal hypertension

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Parker, Sheri B., Anca D. Dobrian, Suzanne S. Wade, and Russell L. Prewitt. AT$_1$ receptor inhibition does not reduce arterial wall hypertrophy or PDGF-A expression in renal hypertension. Am. J. Physiol. Heart Circ. Physiol. 278: H613–H622, 2000.—To separate the role of ANG II from pressure in hypertrophy of the vascular wall in one-kidney, one-clip (1K1C) hypertension, experimental and sham-operated rats were given the AT$_1$-receptor antagonist losartan (20 mg·kg$^{-1}$·day$^{-1}$) or tap water for 14 days. Mean arterial pressure was elevated in both experimental groups compared with controls. Rats were anesthetized with pentobarbital sodium, and the thoracic aorta and carotid, small mesenteric, and external spermatic arteries were harvested and embedded in paraffin. Tissue sections were used for morphological analysis, immunohistochemistry for 5-bromo-2′-deoxyuridine (BrdU) and platelet-derived growth factor (PDGF)-AA, stereological measurements, and in situ hybridization with a $^{35}$S-labeled riboprobe for PDGF-A mRNA. Elevated cross-sectional areas of thoracic, carotid, and small mesenteric artery in 1K1C rats were not reduced by losartan. The internal diameter of the external spermatic artery and microvascular density of the cremaster muscle were reduced in 1K1C rats. The number of BrdU-positive nuclei per cross section did not differ between 1K1C and control arteries. PDGF-A mRNA was elevated in the arterial walls of 1K1C rats compared with controls and was hardly changed by losartan. PDGF-A protein stained strongly in the media of 1K1C arteries and was not inhibited by losartan; it appeared in the adventitia of all aortas and carotid arteries. These observations demonstrate that effects of ANG II mediated through the AT$_1$ receptor are not necessary for hypertrophy of the vascular wall during 1K1C hypertension or expression of PDGF-A.

one-kidney, one-clip hypertension; losartan; arterial pressure; angiotensin; platelet-derived growth factor

DURING THE COURSE of hypertension, individual arteries adapt to mechanical and hormonal stresses through alterations in medial thickness and/or internal and external diameters, depending on the size and function of the particular blood vessel. The larger arteries increase wall cross-sectional area with the development of outward hypertrophy (30, 45). The smaller arterioles undergo a decrease in lumen size without an increase in wall area and/or rarefaction, the reduction in number of functional vessels (16, 31, 38). Lumen reduction in the absence of hypertrophy is termed “inward, eutrophic remodeling.” Both ANG II and pressure have been implicated as stimuli for the vascular changes associated with hypertension (4, 9, 18, 30, 45). ANG II is a hypertrophic (18) as well as hyperplastic stimulus (47) of vascular smooth muscle cells (VSMCs) and has been shown to induce platelet-derived growth factor (PDGF)-A chain expression (28). Recently, we have shown that vascular hypertrophy following chronic infusion of ANG II was entirely prevented when the blood pressure was kept from rising by the simultaneous administration of minoxidil (34). Pressure, like ANG II, is also a hypertrophic stimulus (20, 30) and is correlated with PDGF-A chain expression (6).

Additional studies have implicated ANG II as a mediator of angiogenesis in the microcirculation (17). However, contrary to the effects of ANG II, pressure elevations induce a reduction in the number of vessels rather than an increase (16, 35, 38). As a result of the effect of ANG II on blood pressure, it is often difficult to characterize its direct and indirect effects on the vascular changes associated with hypertension.

In vivo studies that provide evidence that ANG II acts as a growth factor through a non-pressor mechanism include those using angiotensin-converting enzyme inhibitors. A non-depressor dose of captopril during renal hypertension significantly attenuated the medial-intimal area of the abdominal aorta, suggesting a non-pressor mechanism for ANG II (45). However, the use of converting enzyme inhibitors (CEI) as a tool to determine the role of ANG II in hypertrophy is confounded by the accumulation of bradykinin, whose products have antiproliferative effects in vascular smooth muscle (8). These effects were revealed by studies utilizing the bradykinin receptor antagonist icatibant in combination with CEI therapy. Icatibant blocked the attenuation of growth in myocardial mass during CEI therapy, suggesting that CEI is not an effective means for evaluating the role of ANG II alone on vascular growth (23). Therefore, experiments using CEI to assess the direct effects of ANG II on vascular hypertrophy are not conclusive.

ANG II receptors are divided into two distinct categories, type 1 (AT$_1$) and type 2 (AT$_2$). For the most part, the known functions of ANG II are linked to the AT$_1$ receptor, the predominant receptor in adult vascular smooth muscle and kidney (42). The AT$_2$ receptor is dominant in the rat aorta during embryonic stages of development, but this is reversed 8 wk after parturition, when the dominant ANG II aortic receptor be-

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becomes the AT$_1$ subtype (35). AT$_1$ receptor are thought to mediate the growth-promoting effect of ANG II, whereas the AT$_2$ receptor may have antiproliferative properties on cultured endothelial cells (41) and microvessels of the rat cremaster muscle (11). In addition, the AT$_2$ receptors have been shown to mediate vasodilation of afferent arterioles in the kidney (7). The receptor antagonist losartan, specific for the AT$_1$-receptor subtype, allows for evaluation of ANG II-induced alterations in the absence of bradykinin accumulation.

The purpose of this study was to investigate the role of ANG II, through the AT$_1$ receptor, on the vascular changes associated with renal hypertension. This was accomplished through the administration of the AT$_1$-receptor inhibitor losartan to the one-kidney, one-clip (1K1C) hypertensive rat, a model in which ANG II, possibly of vascular origin, is reputed to contribute to vascular hypertrophy (50). We then evaluated alterations including arterial hypertrophy, modifications in PDGF-A expression, and microvascular rarefaction, which are typically encountered during hypertension.

**METHODS**

**Treatment groups.** Male Wistar rats (162–242 g body wt) were randomly divided into four groups: uninephrectomized control [one-kidney, no-clip (IKNC), n = 10]; uninephrectomized control with losartan (IKNC-Los, n = 9); 1K1C hypertension (1K1C, n = 8); and 1K1C hypertension with losartan (1K1C-Los, n = 9). IKNC-Los and 1K1C-Los groups were given losartan fresh in their drinking water at a concentration of 120 mg/l, beginning 1 day before surgery and continuing until the end of the experiment. On the basis of average daily fluid consumption and preliminary studies, this concentration of losartan results in a dose of ~20 mg·kg$^{-1}$·day$^{-1}$.

All rats were anesthetized with a single intraperitoneal injection of ketamine hydrochloride (80 mg/kg) and xylazine (12 mg/kg). Through a midline laparotomy, hypertensive rats were produced by the removal of the right kidney along with the placement of a silver clip with a 230-µm gap on the left renal artery. Control animals were uninephrectomized, and the left renal artery was isolated in the same manner as the hypertensive animals without applying the clip. To minimize the risk of infection, penicillin G (25,000 units) was injected intramuscularly before surgery. The experimental protocol was approved by the institutional animal care and use committee. The rats were fed Teklad rat chow and tap or bottled water ad libitum and maintained on a 12:12-h light-dark cycle for 2 wk.

To ascertain the proliferative response of smooth muscle cells, 5-bromo-2’-deoxyuridine (BrdU; Sigma Chemical, St. Louis, MO), a thymidine analog, was utilized to label nuclei undergoing DNA replication. BrdU was injected at a dose of 100 µg/kg subcutaneously and 30 µg/kg intraperitoneally 18 h before rats were killed, with another 30 µg/kg intraperitoneally injected 6 h thereafter (25).

**Systolic blood pressures.** To assess the development of hypertension, indirect systolic tail-cuff blood pressures were routinely obtained with a Narco Bio-Systems Electro-Sphygmomanometer (Houston, TX) after the rats were warmed at 35°C for 5 min. The mean of three measurements was recorded from each rat during trial periods before surgery and then every other day until the end of the experiment. 1K1C rats whose blood pressure was not elevated >150 mmHg by day 8 were eliminated from the study (n = 1).

**Tissue preparation.** After 14 days of treatment, the animals were anesthetized with pentobarbital sodium (60 mg/kg). The tail artery was cannulated (PE-10) for measurement of mean blood pressure, and heparin (0.1 ml/100 g (1,000 units/ml)) was injected intra-arterially. To confirm ANG II blockade, 25 ng/kg ANG II (Sigma) dissolved in 0.9% saline were slowly injected through a jugular cannula (PE-10) and the change in pressure was recorded. Before euthanasia, the right cremaster muscle was dissected and excess fascia removed. Two circular sections, 0.9 mm in diameter, were removed and placed in 0.25% buffered Formalin and stored at 4°C for future staining with Griffonia simplicifolia I lectin (Sigma). The chest was opened, and the inferior vena cava was cut to allow free flow of venous return. The animal was then perfused through the aorta from a catheter inserted in the apex of the left ventricle with a vasodilator solution of 10$^{-4}$ M sodium nitroprusside, 10$^{-4}$ M papaverine, and 10$^{-4}$ M verapamil in 0.9% saline until cleared of blood. The animal was then perfused at 60 mmHg with 10% buffered Formalin for 5–10 min.

The thoracic aorta, carotid artery, small mesenteric artery, and the distal portion of the external spermatic artery (the feeding arteriole to the cremaster muscle) were dissected free from the surrounding tissues and were immersion-fixed in 10% buffered Formalin for 5–10 days. The thoracic aorta was processed through graded alcohol solutions, and embedded in paraffin.

**Morphological analysis.** Sections (4 µm) of the paraffin-embedded tissues were stained with toluidine blue to visualize the intima-media of the blood vessel. A video-based image system with edge-tracking software (JAVA, Jandel Scientific, San Rafael, CA) was used to measure internal and external circumferences from which internal diameter and intimal-medial area were calculated.

**Stereological measurements.** The procedures for microvascular density measurements were adapted from Hansen-Smith et al. (14). Two circular sections, 0.9 mm in diameter, were removed from the right cremaster muscle, and excess fascia was removed. Sections were rinsed in saline and immersion-fixed in 0.25% Formalin for 1–7 days at 4°C. The basement membranes of microvessels were then stained by immersing the tissue in 10 µg/ml rhodamine-labeled Griffonia simplicifolia I lectin (Becton) for 3 h and immediately rinsing with saline. This was followed by 10- and 30-min saline rinses. Tissues were blotted dry and mounted on slides utilizing S/P AccuMount 280 Mounting Medium (VWR Scientific, Bridgeport, NJ). With the use of an Olympus BH-2 series microscope and a rhodamine filter, stereological measurements were performed using a 10 × 10 eyepiece grid to count the capillary intersections at ×300 magnification. Each grid square was 55 µm wide with respect to the microcirculation. Two slides of each muscle were studied, and three fields from each slide were randomly selected and counted. The results of the six fields were averaged to give a single microvascular density value for each muscle.

**Immunohistochemistry for BrdU.** Incorporation of BrdU in the nuclei, a marker for DNA replication during the infusion period, was visualized using a previously published protocol (34). Sections from paraffin-embedded tissues were deparaffinized, rehydrated, blocked for endogenous peroxidases, treated with 2 N HCl for 30 min to denature DNA, enzymatically pretreated with trypsin, and then incubated with 5% normal goat serum. The sections were incubated with mouse monoclonal anti-BrdU (diluted 1:400), and negative controls incubated with 5% normal goat serum were processed in parallel. All slides were incubated with biotinylated secondary antibody IgG (diluted 1:500), stained with a Vectastain Elite avidin-biotin complex (ABC) kit, and incubated with
0.1% daminobenzene solution. To confirm that all the animals received BrdU, the kidneys from each animal were tested for BrdU. Rats with negative kidneys were eliminated (n = 3).

Immunohistochemistry for PDGF-A. Sections of aorta and cardiac arteries were deparaffinized in xylene and rehydrated in graded ethanol. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide in cold methanol, and nonspecific binding was blocked by incubation in 5% normal goat serum in PBS, supplemented with 1% BSA. The sections were then incubated with a polyclonal rabbit anti-human PDGF-AA antibody (Genzyme Diagnostics, Cambridge, MA) that recognizes both human and rat (22, 37) PDGF-A chain (in the form of both AA and AB dimers) and demonstrates <10% reactivity with PDGF-BB. The primary antibody was used at a 1:75 dilution in 1% BSA-PBS overnight, at 4°C, in a humid chamber. After successive washings in PBS and BSA-PBS between each incubation, the slides were reacted with biotinylated secondary goat anti-rabbit antibody (1:500 dilution; Vector Laboratires, Burlingame, CA), the ABC-Elite avidin reagent (Vector Laboratories), and, finally, with the Immuno Pure Metal Enhanced DAB Substrate kit (Pierce, Rockford, IL) as the chromogen. The sections were counterstained with Gill’s hematoxylin, dehydrated, and covered with 10% glycerol.

In situ hybridization and quantification. Expression of PDGF-A mRNA was determined by a previously published (34) in situ hybridization technique based on the method of Wilcox (48). Paraffin sections of arteries were mounted on Superfrost/Plus slides. 35S-labeled sense and antisense riboprobes for PDGF-A chain were transcribed from a 368-bp restriction fragment of the cDNA inserted into the polycloning site of pBluescript SK(+). Briefly, slides were deparaffinized by two washes in xylene. The vessels were then rehydrated in graded alcohol and washed with 0.5× sodium citrate-sodium chloride (SSC). Vessels were then treated with proteinase K at room temperature, washed three times with PBS, and fixed in 4% paraformaldehyde for 15 min at 4°C. Slides were then washed three times with PBS. The vessel sections were then covered with 200 µl of prehybridization solution (10% dextran sulfate, 1× Denhardt solution, 1 mM EDTA, 10 mM Tris, 0.3 M NaCl, 50% formamide, 0.5 mg/ml yeast tRNA, and 10 mM dithiothreitol) and incubated for 3 h at 38–42°C in a humidified chamber. Slides were then covered with 100 µl of hybridization solution (prehybridization solution containing 107 counts/min of probe per ml) and incubated overnight at 55°C in a humidified chamber. Slides were then washed with 2× SSC containing 1 mM EDTA and 10 mM β-mercaptoethanol and treated with ribonuclease A (30 µg/ml) for 30 min at room temperature. Slides were then washed with 2× SSC containing 1 mM EDTA and 10 mM β-mercaptoethanol four times for 30 min, followed by washing with 0.5× SSC. Finally, the vessel sections were dehydrated in graded alcohol solutions and air-dried.

Results were quantified by densitometric analysis with a Molecular Dynamics PhosphorImager SF (Sunnyvale, CA). Slides were exposed on the PhosphorImager cassette for 5 days. With the use of a volume integration function, the total density minus the background was determined for each vessel cross section. The mean value in arbitrary units was calculated to obtain a single value for each vessel. Sense probe binding of all groups was either very low or undetectable. The density counts for binding of sense probe were then subtracted from the counts for binding for antisense probes to determine the mRNA levels for PDGF-A.

Materials. The cDNA for rat PDGF-A was obtained from Tucker Collins at Brigham & Women’s Hospital (Boston, MA). Radiolabeled cytidine triphosphate was obtained from DuPont (Boston, MA). Losartan was a gift from Ron D. Smith at DuPont Merck Research and Development (Wilmington, DE). All other chemicals or biochemicals were obtained from Sigma Chemical (St. Louis, MO), Fischer Scientific (Pittsburgh, PA), GIBCO BRL (Gaithersburg, MD), or Promega (Madison, WI).

Statistics. Results are expressed as means ± SE. Statistical analyses were performed using ANOVA, and the null hypothesis was rejected at P < 0.05.

RESULTS

1K1C-Los animals ingested 20.8 ± 0.8 mg·kg⁻¹·day⁻¹ of losartan, whereas 1KNC-Los animals ingested 17.8 ± 0.7 mg·kg⁻¹·day⁻¹. Tail systolic blood pressures were significantly elevated in both 1K1C and 1K1C-Los animals (Fig. 1) above those of the 1KNC and 1KNC-Los groups beginning 4 days after surgery (day 0) and continuing for the duration of the study. There were significant differences between the 1K1C and 1K1C-Los groups on day 6 and day 12 of the study, but because these differences reversed direction, they have little physiological significance. Losartan lowered the systolic blood pressure in 1KNC-Los rats from day 8 through day 12 compared with the 1K1C group.

Mean arterial blood pressures measured through an indwelling catheter in the tail artery were significantly elevated in clipped animals (1K1C, 151.3 ± 9.1 mmHg; 1K1C-Los, 149.7 ± 12.9 mmHg) compared with the uninephrectomized control groups (1KNC, 112.3 ± 6.3 mmHg; 1KNC-Los, 102.8 ± 6.9 mmHg). Injection of 25 ng/kg ANG II in the losartan-treated animals produced...
a blunted pressor response (1K1C-Los, $-0.5 \pm 0.8$ mmHg; 1KNC-Los, $-3.67 \pm 1.6$ mmHg) compared with groups given tap water (1K1C, $28.5 \pm 6.7$ mmHg; 1KNC, $36.5 \pm 9.3$ mmHg). Body weights of all four groups were similar ($P > 0.05$).

Cross-sectional wall area was significantly elevated in the thoracic aorta, carotid artery, and small mesenteric artery of the 1K1C animals, and these results were not affected by treatment with losartan (Fig. 2). The 1KNC-Los animals had a significant decrease in cross-sectional wall area in the thoracic aorta and carotid artery compared with the 1KNC animals. There were no significant differences in cross-sectional wall area of the external spermatic artery. The internal diameter of the aorta of 1K1C-Los animals was significantly greater than that of the 1KNC-Los animals (Fig. 3); otherwise, there were no differences among the groups in the internal diameter of the aorta and carotid and small mesenteric arteries. The internal diameter of the external spermatic artery was significantly reduced in the 1K1C animals compared with the 1KNC and 1KNC-Los animals.

Staining for BrdU was low in the carotid and small mesenteric arteries of all the groups ($P > 0.05$). The only significant difference was between the positively stained nuclei in the thoracic aorta of 1K1C-Los (1.6 $\pm 0.4$ nuclei) and 1KNC-Los (0.2 $\pm 0.1$ nuclei) groups, values that were not different from those of the 1K1C (0.5 $\pm 0.2$ nuclei) and 1KNC (1 $\pm 0.3$ nuclei) groups. There was no positively staining tissue in the external spermatic arteries in any of the four groups. All kidneys with the exception of those from three animals had positively staining nuclei in the renal tubules, confirming BrdU delivery through injection. Arteries from the three negative animals were excluded from BrdU analysis.

Staining with *Griffonia simplicifolia* I lectin, which binds to the basal membrane of the microvessels, showed that 1K1C hypertension reduced capillary density by $-22\%$ compared with both uninephrectomized controls (Fig. 4). Density in 1K1C-Los animals was intermediate and not significantly different from any of the other values.

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Fig. 2. Cross-sectional wall area of thoracic aorta (A), carotid artery (B), small mesenteric artery (C), and external spermatic arteriole (D) for uninephrectomized controls (1KNC), hypertensive (1K1C), control losartan-treated (1KNC-Los), and hypertensive losartan-treated (1K1C-Los) groups. Wall area was determined with a video-based image system using edge-tracking software on toluidine blue-stained tissues. Values are means $\pm$ SE. *$P < 0.05$ vs. 1KNC rats. + $P < 0.05$ vs. 1KNC-Los rats.

Fig. 3. Internal diameter of thoracic aorta (A), carotid artery (B), small mesenteric artery (C), and external spermatic arteriole (D) for 1KNC, 1K1C, 1KNC-Los, and 1K1C-Los groups. Internal diameter was determined with a video-based image system using edge-tracking software on toluidine blue-stained tissues. Values are means $\pm$ SE. *$P < 0.05$ vs. 1KNC. + $P < 0.05$ vs. 1KNC-Los rats.
Representative phosphorimages of thoracic aortas from a 1K1C rat hybridized to a sense and an antisense 35S-labeled riboprobe for PDGF-A mRNA are shown in Fig. 5. Expression of PDGF-A mRNA was significantly elevated in the thoracic aorta of both the 1K1C and 1K1C-Los groups compared with the 1KNC group (Fig. 6). In the carotid artery, PDGF-A mRNA expression was significantly elevated in 1K1C and 1K1C-Los groups compared with both control groups. Expression of PDGF-A mRNA was significantly elevated in the small mesenteric arteries of the 1K1C animals compared with both control groups, and treatment with losartan blunted the response.

As shown in Figs. 7 and 8, the mRNA for PDGF-A was translated into higher protein levels in the media of the 1K1C aorta and carotid arteries, and losartan did not prevent this expression. Aortas and carotid arteries of both 1K1C and 1KNC showed positive staining for PDGF-A in the adventitia, but this was very faint in the mesenteric arteries.

DISCUSSION

The renin-independent 1K1C hypertensive rat (27) was used in this study because the effects of ANG II can be inhibited without changing arterial pressure in this model. Thus the effects of elevated blood pressure on vascular remodeling were left intact while the possible contribution of ANG II through the AT1 receptor was blocked by the administration of losartan. Renal artery stenosis induced a significant rise in blood pressure beginning 4 days after surgery and continuing for the duration of the study (Fig. 1). Treatment with losartan did not prevent the rise in blood pressure. AT1 receptor blockade was confirmed in the losartan-treated groups by the lack of pressor response to a bolus injection of ANG II. Therefore, this experimental model allowed for the separation of the effects of ANG II, through the AT1 receptor, and elevated arterial pressure.

Cross-sectional wall area in the thoracic aorta and carotid and small mesenteric arteries was significantly increased in 1K1C hypertensive rats (Fig. 2).
increase was not affected by treatment with losartan, implying that pressure rather than ANG II is the major contributor to arterial hypertrophy in this particular model of hypertension. There is considerable evidence that pressure is capable of eliciting the structural changes associated with hypertension. Experiments on aortic coarctation demonstrated an increase in cross-sectional wall area in only the hypertensive region of the animal (21, 30). In addition, elevated blood pressure is correlated with increased wall-to-lumen ratio in mesenteric arteries of sodium-loaded spontaneously hypertensive rats (SHR) during CEI therapy, implying that pressure is the stimulus for these vascular alterations (15). Interestingly, the 1KNC-Los group in the present study demonstrated a significant decrease in cross-sectional wall area in the thoracic aorta and carotid artery compared with the 1KNC group. This may be attributable to the difference in systolic blood pressure between the two groups for almost one-half of the duration of the study.

Corroborating evidence for pressure as a stimulus for arterial hypertrophy was obtained in chronic ligation experiments, in which protecting the vascular bed from elevated pressure in DOCA-salt hypertensive rats and SHR resulted in a medial thickness unchanged from that of normotensive controls (13). Similar ligation experiments in the SHR by Bund et al. (4) and Folkow et al. (9) produced the same results. There are, however, experiments suggesting that ANG II contributes to arterial hypertrophy through a non-pressor mechanism. CEI treatment in 1K1C hypertension resulted in a significant decrease in aortic wall area without a decrease in arterial pressure (45). As stated earlier, however, experiments suggesting that ANG II contributes to arterial hypertrophy through a non-pressor mechanism, CEI treatment in 1K1C hypertension resulted in a significant decrease in aortic wall area without a decrease in arterial pressure (45). As stated earlier, however, these results were confounded by the potentiation of bradykinin during CEI. Another study, utilizing ANG II receptor antagonism in SHR, demonstrated a

![Fig. 7. Representative micrographs showing immunostaining for PDGF-A in thoracic aortas (A–D) or carotid arteries (E–H). Vessels from both 1K1C (A and E) and 1K1C-Los (B and F) animals show an evenly distributed positive staining for PDGF-A in medial layer as well as in adventitia. Vessels from 1KNC (C and G) and 1KNC-Los (D and H) animals exhibit very few cells stained in media and some staining in adventitial layer. Note that losartan treatment did not prevent enhanced PDGF-A protein expression in hypertensive animals. Bar, 50 µm.](image)

![Fig. 8. Representative micrographs showing immunostaining for PDGF-A in mesenteric arteries of 1K1C (B), 1K1C-Los (C), 1KNC (E), and 1KNC-Los (F) rats. Method controls lacking primary antibody are shown for 1K1C (A) and 1KNC (D) rats. Unlike aorta and carotid arteries, there is little staining in adventitia of either hypertensive or control mesenteric arteries. Bar, 50 µm.](image)
significantly decreased media-to-lumen ratio in the small mesenteric artery (43). This decreased ratio, however, was accompanied by a significant decrease in systolic blood pressure that correlated with the arterial morphological changes, implicating pressure as a mediator of the response. Together with these published results, the present study provides convincing evidence that regulation of arterial hypertrophy in renal hypertensive animals during AT₁ receptor blockade is mediated by elevated blood pressure.

The external spermatic artery, also known as the first-order arteriole of the cremaster muscle, did not hypertrophy in the hypertensive animals, regardless of drug treatment. Yet there was a significant decrease in the internal diameter of this artery in the 1K1C group (Fig. 3). In hypertension, this characteristic structural change, known as inward, eutrophic remodeling, occurs in vessels <150 µm in diameter (16, 38). Pressure has been implicated as the major stimulus for this remodeling because of its failure to take place in cremaster arterioles of rats subjected to aortic coarctation (40), in which only the upper body is hypertensive. Recent experiments from our laboratory have shown that a pressure stimulus activates protooncogenes in isolated resistance arteries (1) and that the gene expression correlates with wall stress rather than cellular stretch (2). The lack of hypertrophy in the smaller arteries during hypertension may be explained by the law of Laplace, in which wall stress is directly proportional to the product of pressure and radius and inversely proportional to wall thickness. The increase in tone of these vessels during the initiation (24) and development of renal hypertension (36) will decrease the radius and oppose any increase in wall stress. The large arteries, which have little tone, control their wall stress by the increase in wall thickness. Thus pressure can be the stimulus for both hypertrophic and eutrophic remodeling. The two different forms of remodeling cannot be explained as easily by ANG II or any other circulating growth factor.

Typically, the mechanism of increased cross-sectional wall area in the larger arteries is through VSMC hypertrophy with or without polyploidy (20, 33). The results of this study demonstrate little or no staining for BrdU in all of the carotid and small mesenteric arteries, implying that the increased cross-sectional wall area in clipped animals was indeed caused by cellular hypertrophy. There was a small but physiologically insignificant increase in BrdU incorporation in the aortic VSMC of the losartan-treated controls compared with the 1K1C-Los group, but there was no difference between the untreated controls and either hypertensive group. Thus cellular hypertrophy also accounts for the increase in aortic wall thickness.

Although pressure is thought to mediate this response, several lines of evidence suggest that ANG II is also a plausible factor in the hypertrophy of VSMC. Application of the hormone to VSMCs (10) and intact aortic rings induces an increase in protein content but not DNA synthesis (18). In addition, CE1 treatment of SHR and Wistar-Kyoto (WKY) rats reduces polyploidy of aortic VSMCs beyond that expected from the decrease in pressure (32). However, the present results showing a lack of any effect of AT₁ blockade in the hypertensive arteries suggest that pressure mediated the hypertrophic response of the VSMC.

The results for microvascular density (Fig. 4) show the typical rarefaction in the 1K1C group and an attenuation of rarefaction by treatment with losartan. At present, there are two hypotheses to explain microvascular rarefaction during hypertension. One hypothesis suggests that microvascular density is controlled by the balance of AT₁ and AT₂ receptors, with the AT₁ subtype promoting microvascular growth and the AT₂ subtype inhibiting growth (26). Feeding rats a high-salt diet produces microvascular rarefaction that can be prevented by infusion of small doses of ANG II (17). AT₂ receptor stimulation has been shown to inhibit the growth of cultured coronary endothelial cells (41), and if this occurred in vivo, it could inhibit the growth of capillaries. AT₂ receptor activation also leads to apoptosis under certain conditions (5), and this could be a mechanism for microvascular rarefaction. If this hypothesis were correct, blockade of the AT₁ receptors in the present experiment would leave the AT₂ receptors free to maximize the amount of rarefaction. In addition, the concentration of ANG II may be greatly elevated during treatment with losartan, which increases plasma renin activity more than eightfold in 1K1C rats (unpublished observations, n = 3). Because losartan attenuated the amount of rarefaction rather than increasing it, this hypothesis is not consistent with the data unless some other factor is postulated, such as downregulation of AT₂ receptors when losartan is given.

The other hypothesis to account for microvascular rarefaction is that it is a chronic autoregulatory mechanism to control blood flow through changes in vascular density. Hypertension is the opposite of a reduction in perfusion pressure that has been shown to increase the growth of microvessels (44). In support of this hypothesis are the data showing increased arteriolar tone and an increased number of closed arterioles, or functional rarefaction, preceding the development of structural rarefaction in this 1K1C model of hypertension (16) as well as the SHR (35). Vasoconstriction by ANG II is mediated through the AT₁ receptor, and it is consistent with the autoregulatory hypothesis that AT₁ receptor inhibition would decrease arteriolar tone and reduce functional and then structural rarefaction (Fig. 4).

Another finding in this study is the significant increase in PDGF-A expression in the wall of the hypertrophied aorta and carotid and small mesenteric arteries that, with the exception of the small mesenteric arteries, was unaffected by losartan (Fig. 6). As is the case for hypertrophy itself, PDGF-A expression can be attributed to elevated pressure or stimulation of the VSMCs by hormonal factors including ANG II. Previous work from this laboratory showed that PDGF-A expression and vascular wall hypertrophy both correlate with blood pressure in the 1K1C hypertensive rat (6). Decreasing blood pressure by different antihypertensive agents in the SHR also produced a decrease in
PDGF-A expression and aortic hypertrophy, both of which correlated with the change in blood pressure (29). These results from two different models of hypertension suggest that pressure mediates PDGF-A expression in the arterial VSMCs. Cyclic stretch of VSMCs grown on fibronectin elicits PDGF-A synthesis (49), showing that the response occurs independently of neural or hormonal influence.

ANG II, nonetheless, is also implicated as a stimulus for PDGF-A expression in cell culture (28) as well as in vivo by ANG II infusion (12, 39, 46). Our recent work has shown that the hypertrophic response is mediated by the elevation in blood pressure when ANG II is kept within physiological levels (34). Collectively, these results suggest that the increase in PDGF-A expression is mediated by pressure.

Previous studies in our laboratory demonstrated that PDGF-A chain but not -B chain was elevated in the aortic wall in 1K1C hypertension (6). There are significant differences between the proliferative effects of the dimeric chains on VSMCs; PDGF-BB and -AB promote DNA synthesis (25), whereas PDGF-AA is a weak mitogen (19). In cell culture, PDGF-BB and -AB chains induce DNA synthesis in a concentration-dependent manner in both WKY and SHR VSMCs, whereas a larger concentration of PDGF-AA chains weakly induced proliferation only in SHR VSMCs (3). Therefore, it is suggested that the increased expression of each respective PDGF chain, depending on its mitogenic properties, may be a predictor of whether or not the smooth muscle cell undergoes a proliferative response. The present study demonstrates a significant increase in PDGF-A expression and vascular hypertrophy without accompanying DNA synthesis. These results, combined with evidence from previous studies, are consistent with the hypothesis that pressure-induced changes in vascular hypertrophy are mediated in part by an increase in PDGF-A expression.

In summary, the results of these studies indicate, first, that ANG II, acting through the AT1 receptor, is not necessary for vascular hypertrophy in 1K1C hypertension, implying that pressure is the mediator of this response. Second, AT1 receptor inhibition does not decrease capillary density in contrast to the hypothesis that AT1 receptors stimulate microvascular growth and AT2 receptors inhibit it. Third, the results suggest that PDGF-A expression appears to be related primarily to elevated pressure rather than AT1 receptor stimulation.

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