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Localization and differential regulation of angiotensinogen mRNA expression in the vessel wall.

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Research Article

Recent data demonstrate the existence of a vascular renin angiotensin system. In this study we examine the localization of angiotensinogen mRNA in the blood vessel wall of two rat strains, the Wistar and Wistar Kyoto (WKY), as well as the regulation of vascular angiotensinogen mRNA expression by dietary sodium. Northern blot analysis and in situ hybridization histochemistry demonstrate that in both strains angiotensinogen mRNA is detected in the aortic medial smooth muscle layer as well as the periaortic fat. In WKY rats fed a 1.6% sodium diet, angiotensinogen mRNA concentration is 2.6-fold higher in the periaortic fat than in the smooth muscle, as analyzed by quantitative slot blot hybridization. Angiotensinogen mRNA expression in the medial smooth muscle layer is sodium regulated. After 5 d of a low (0.02%) sodium diet, smooth muscle angiotensinogen mRNA levels increase 3.2-fold (P less than 0.005) as compared with the 1.6% sodium diet. In contrast, angiotensinogen mRNA level in the periaortic fat is not influenced by sodium diet. In summary, our data demonstrate regional (smooth muscle vs. periaortic fat) differential regulation of angiotensinogen mRNA levels in the blood vessel wall by sodium. This regional differential [...]

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Localization and Differential Regulation of Angiotensinogen mRNA Expression in the Vessel Wall

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Abstract

Recent data demonstrate the existence of a vascular renin-angiotensin system. In this study we examine the localization of angiotensinogen mRNA in the blood vessel wall of two rat strains, the Wistar and Wistar Kyoto (WKY), as well as the regulation of vascular angiotensinogen mRNA expression by dietary sodium. Northern blot analysis and in situ hybridization histochemistry demonstrate that in both strains angiotensinogen mRNA is detected in the aortic medial smooth muscle layer as well as the periaortic fat. In WKY rats fed a 1.6% sodium diet, angiotensinogen mRNA concentration is 2.6-fold higher in the periaortic fat than in the smooth muscle, as analyzed by quantitative slot blot hybridization. Angiotensinogen mRNA expression in the medial smooth muscle layer is sodium regulated. After 5 d of a low (0.02%) sodium diet, smooth muscle angiotensinogen mRNA levels increase 3.2-fold ($P < 0.005$) as compared with the 1.6% sodium diet. In contrast, angiotensinogen mRNA level in the periaortic fat is not influenced by sodium diet. In summary, our data demonstrate regional (smooth muscle vs. periaortic fat) differential regulation of angiotensinogen mRNA levels in the blood vessel wall by sodium. This regional differential regulation by sodium may have important physiological implications. (*J. Clin. Invest.* 1991. 87:1300-1311.) Key words: medial smooth muscle • angiotensin • vascular renin • angiotensin • sodium

Introduction

In recent years the existence and the potential importance of the local renin-angiotensin system in various tissues (e.g., the blood vessel) has begun to be appreciated (1). A recent report by Okamura et al. (2) suggests that an increase in vascular angiotensin activity may, in part, be responsible for the chronic hypertension in the two-kidney, one-clip rat. The importance of this local renin-angiotensin system is further supported by the reports that the depressor response to angiotensin convert-

ing enzyme (ACE)¹ inhibitors correlates better with the inhibition of ACE in vascular tissue than with the inhibition of serum ACE activity (3, 4).

With the availability of a specific cDNA probe, a number of investigators have demonstrated the presence of angiotensinogen mRNA in a variety of tissues including kidney, brain, heart, adrenal gland, and blood vessels (5-10). Campbell and Habener (6) quantitated the amount of angiotensinogen mRNA in various rat tissues and reported that the concentration in the aorta was approximately one-third the level present in the liver, and was significantly higher than that found in the kidney, heart, adrenal, or brain. Based on an in situ hybridization study of the aorta from rats treated with estrogen, thyroxine, and dexamethasone, they reported that angiotensinogen mRNA was highly abundant in the periaortic brown adipose tissue (11). Using Northern blot analysis, Cassis et al. (12) studied the Sprague Dawley rat under uncontrolled conditions and found angiotensinogen mRNA to be present primarily in the periaortic fat. In this study, we examined the effect of dietary sodium on the location and level of angiotensinogen mRNA expression in the aorta of the Wistar Kyoto (WKY) rat using both in situ hybridization and Northern blot analysis. Our results demonstrated that angiotensinogen mRNA was present in the medial smooth muscle layer as well as the surrounding fat of the WKY aorta. These findings were confirmed in the aorta of the Wistar rat. In addition, sodium restriction stimulated aortic angiotensinogen mRNA expression in the medial smooth muscle layer of the WKY aorta but not in the periaortic fat. Our data would suggest that the quantitative distribution of angiotensinogen mRNA in the various regions of the aorta is influenced by the physiological condition of the animal.

Methods

Animals and tissue processing

Male Wistar and WKY rats, 10-12 wk old, were purchased from Charles River Breeding Laboratories, Inc., Wilmington, MA, for studies of regional localization of angiotensinogen mRNA expression. To examine the influence of sodium diet on angiotensinogen mRNA expression, WKY rats were maintained on either a 0.02% or a 1.6% sodium diet for 1-5 d. A total of 18 WKY were studied for each diet. Animals were killed by decapitation. For RNA analysis, the thoracic aorta was dissected from the take-off of the left subclavian artery to the diaphragm, and the adventitia and periaortic fat were stripped off by forceps. The vessel was then opened lengthwise and the endothelium scraped away using the blunt end of forceps. Remaining adventitia was

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1. Abbreviations used in this paper: ACE, angiotensin converting enzyme; WKY, Wistar Kyoto.

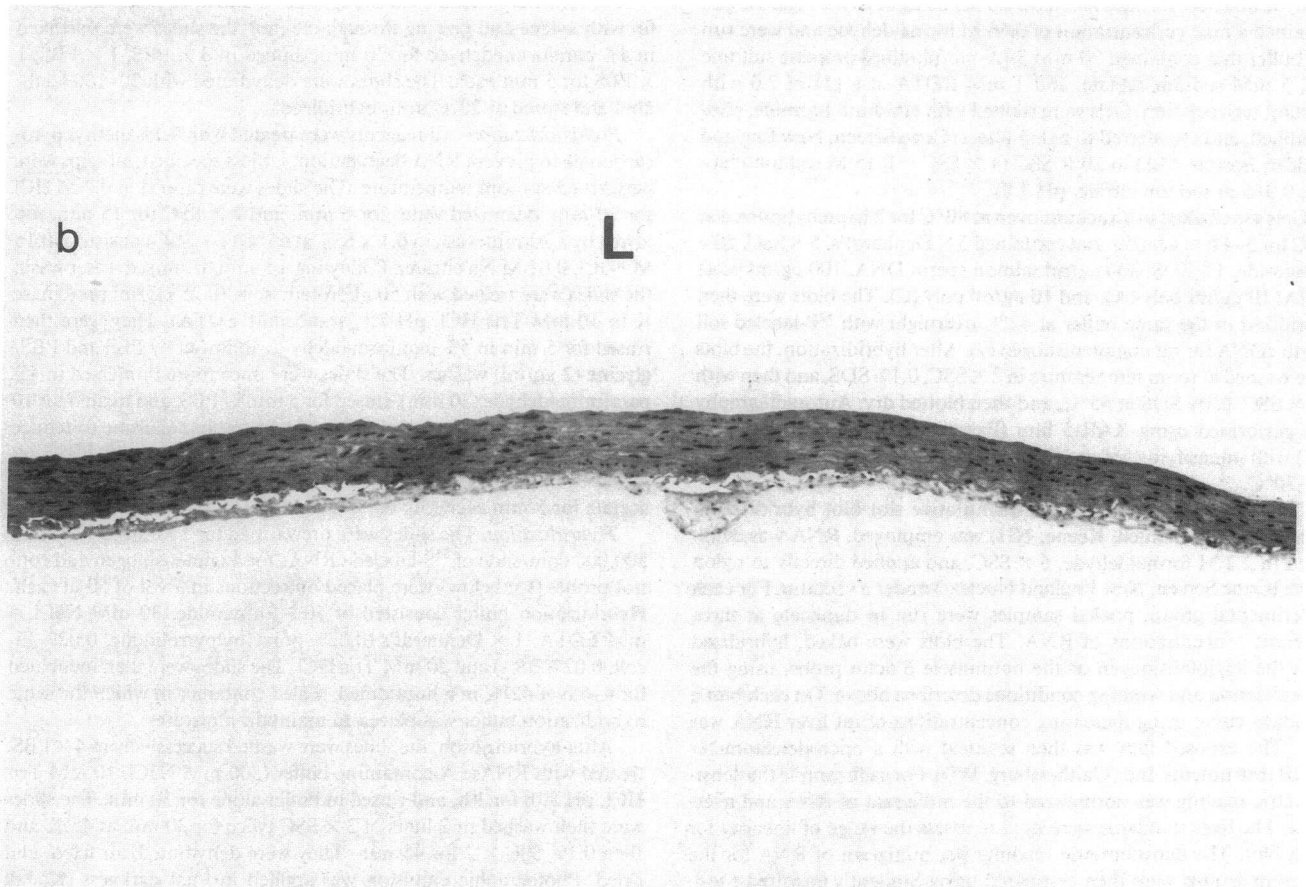
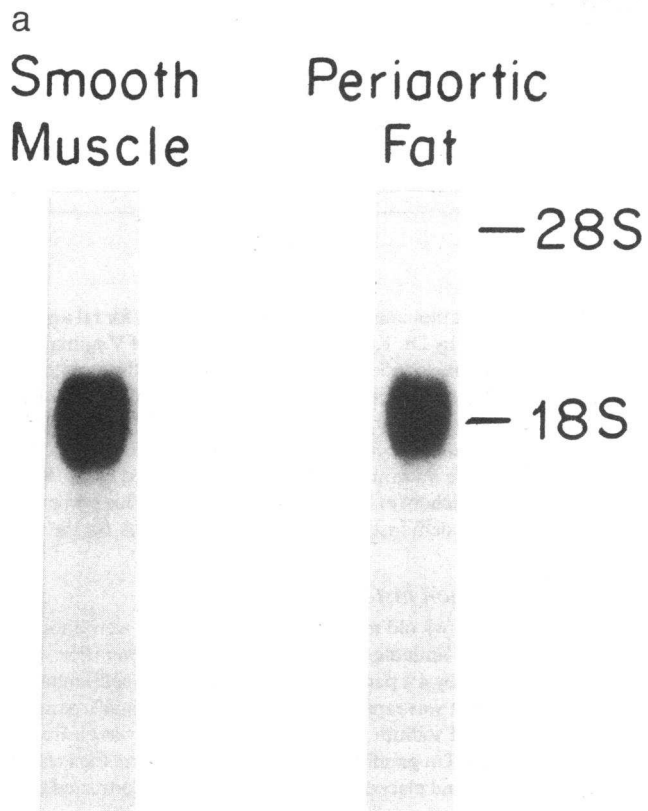


Figure 1. (a) Representative Northern blot of 25 μ g of total RNA from the medial smooth muscle layer (*left lane*) and periaortic fat layer (*right lane*) of WKY rats. Markers on the right show the position of the 28S and 18S ribosomal RNA bands located by ethidium bromide staining. (b) Hematoxylin and eosin staining of a representative section of stripped aorta containing mainly the medial smooth muscle layer. *L* denotes the lumen.

Figure 2. Localization of angiotensinogen mRNA in the Wistar rat aorta by in situ hybridization. *A–C* ($\times 40$) show cross-sections of aorta hybridized, respectively, to radiolabeled angiotensinogen cRNA (antisense probe), control probe (radiolabeled sense probe), and excess cold probe in competition with radiolabeled antisense probe. As can be seen in *A*, the antisense probe hybridizes to the RNA in the aortic media. *D–F* show the same respective sections at $\times 100$ power. Again, only *D* shows specific grains representing angiotensinogen mRNA in smooth cells. *G–I* illustrate periadventitial fat ($\times 100$) hybridized with radiolabeled antisense sense probe (control), and cold antisense probe competed with radiolabeled probe, respectively. Only the radiolabeled antisense probe (*G*) demonstrated specific hybridization in adipose cells. In addition, RNase pretreatment abolished silver grains when used before hybridization (not shown). In each section, 500,000 counts of ^{35}S -UTP-labeled probe was applied. Sections were exposed 5 wk before developing. Staining is with hematoxylin and eosin. *L* denotes the lumen.

stripped away further. The kidneys and livers were also removed simultaneously from the rats. The tissues were quickly frozen in liquid nitrogen and stored at -70°C until use. For morphologic studies, pieces of stripped aorta were fixed in formalin, frozen, cut into thin sections, and stained with hematoxylin and eosin.

RNA isolation

For each extraction, the aortic medial smooth muscle layer from three animals or the adventitia plus periaortic fat from two animals were pooled and homogenized in 7.5 ml of 4 M guanidium thiocyanate, 0.5% sodium *N*-lauryl sarcosine, 25 mM sodium citrate, 2 M cesium chloride, and 0.1 M beta-mercaptoethanol (13). The homogenate was then applied to 5 ml of 5.7 M cesium chloride, 25 mM sodium acetate, pH 5.5, and centrifuged at 35,000 rpm for 16–18 h at 20°C to pellet the RNA. The RNA was resuspended in 0.2 M sodium acetate, ethanol precipitated, resuspended in distilled water, and quantitated by absorbance at 260 nm. The RNA was stored at -70°C for future use.

Northern and slot blot hybridizations

RNA was lyophilized, denatured with formaldehyde, and run on 1.5% agarose gels. 20 μl of a denaturing mixture that consisted of 7.2% formaldehyde and 50% formamide was used per 25 μg of RNA. Agarose gels contained a final concentration of 0.66 M formaldehyde and were run in a buffer that contained 20 mM 3-[*N*-morpholino] propane sulfonic acid, 5 mM sodium acetate, and 1 mM EDTA at a pH of 7.0 with constant recirculation. Gels were stained with ethidium bromide, photographed, and transferred to nylon filters (Gene Screen; New England Nuclear, Boston, MA) in $20 \times \text{SSC}$ ($1 \times \text{SSC} = 0.15 \text{ M}$ sodium chloride, 0.015 M sodium citrate, pH 7.0).

Gels were baked in a vacuum oven at 80°C for 2 h, prehybridized at 42°C for 3–4 h in a buffer that contained $5 \times \text{Denhardt's}$, $5 \times \text{SSC}$, 50% formamide, 1% SDS, 200 $\mu\text{g}/\text{ml}$ salmon sperm DNA, 100 $\mu\text{g}/\text{ml}$ yeast tRNA, 10 $\mu\text{g}/\text{ml}$ poly (A), and 10 $\mu\text{g}/\text{ml}$ poly (C). The blots were then hybridized in the same buffer at 42°C overnight with ^{32}P -labeled full length cDNA for rat angiotensinogen (9). After hybridization, the blots were washed at room temperature in $2 \times \text{SSC}$, 0.1% SDS, and then with $0.2 \times \text{SSC}$, 0.1% SDS at 65°C , and then blotted dry. Autoradiography was performed using XAR-5 film (Eastman Kodak Co., Rochester, NY) with intensifying screens (DuPont Instruments, Wilmington, DE) at -70°C .

To determine mRNA levels, quantitative slot blot hybridization (Schleicher and Schuell, Keene, NH) was employed. RNA was denatured in 2.2 M formaldehyde, $6 \times \text{SSC}$, and applied directly to nylon filters (Gene Screen; New England Nuclear) under a vacuum. For each experimental group, pooled samples were run in duplicate at three different concentrations of RNA. The blots were baked, hybridized with the angiotensinogen or the nonmuscle β actin probe, using the hybridization and washing conditions described above. On each blot a standard curve using increasing concentrations of rat liver RNA was run. The exposed film was then scanned with a microdensitometer (LKB Instruments, Inc., Gaithersburg, MD). For each sample the densitometric reading was normalized to the milligram of RNA and averaged. The liver standards were used to assess the range of linearity for each blot. The densitometric readings per milligram of RNA for the different groups were then compared using Student's unpaired *t* test. Since each blot had identical liver standards, interblot comparisons could be made. Our intrablot and interblot variations in this assay are 7 and 9%, respectively.

Labeling of cDNA

For Northern and slot blot analysis, the full length cDNA for rat angiotensinogen (provided by Dr. Kevin Lynch, University of Virginia) (9) was labeled by oligonucleotide-primed replacement synthesis with 25 μCi of [α ^{32}P] deoxycytidine 5'-triphosphate (1,000–1,500 Ci/mmol, New England Nuclear) as described (14). The labeled cDNA was purified by Sephadex G-50 chromatography. Similarly, a 600-bp fragment of the mouse nonmuscle β actin probe (provided by Dr. Russel Medford, Emory School of Medicine) was prepared. This probe was used as a control to demonstrate equal loading of RNA on the slot blots (8).

In situ hybridization histochemistry

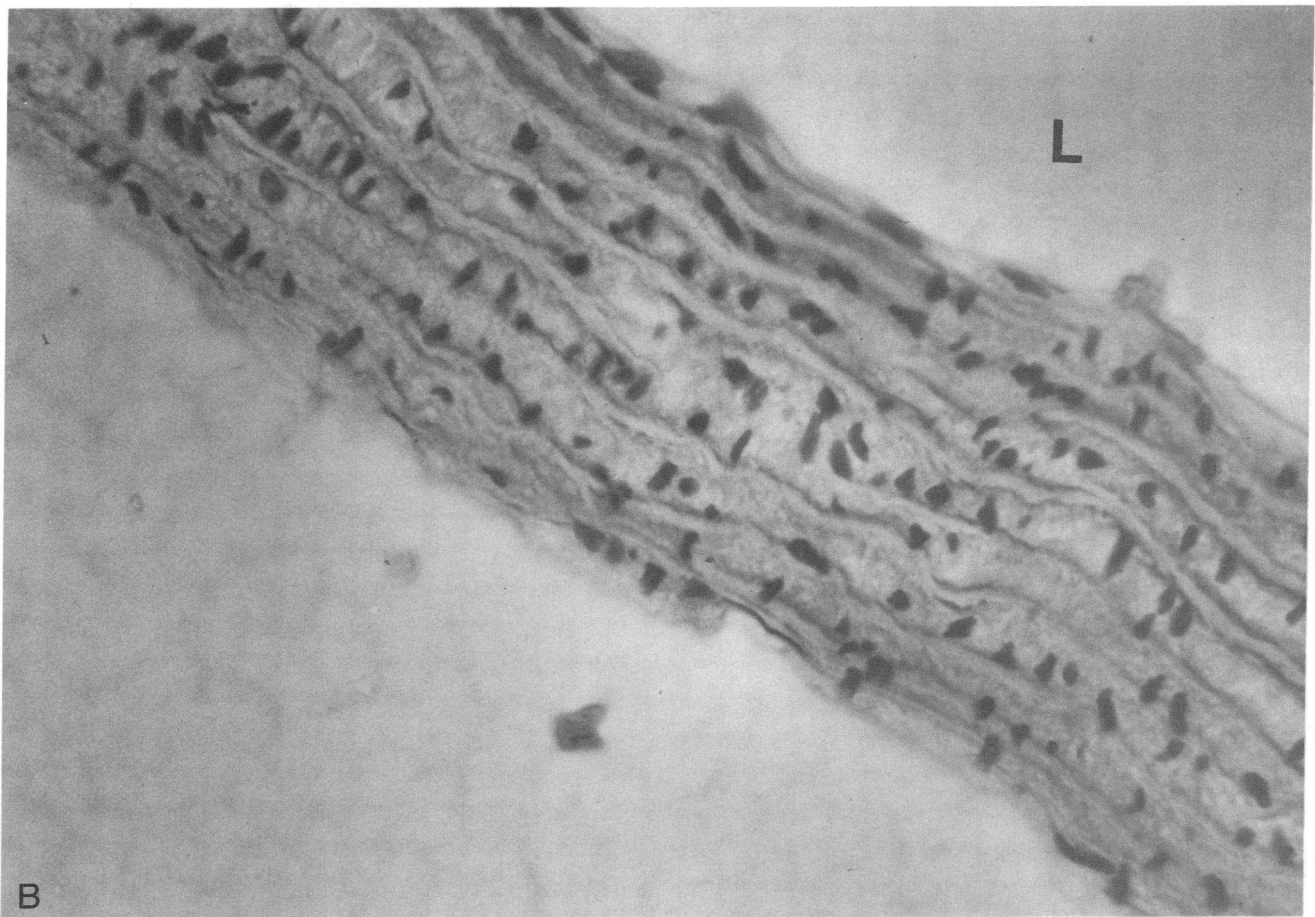
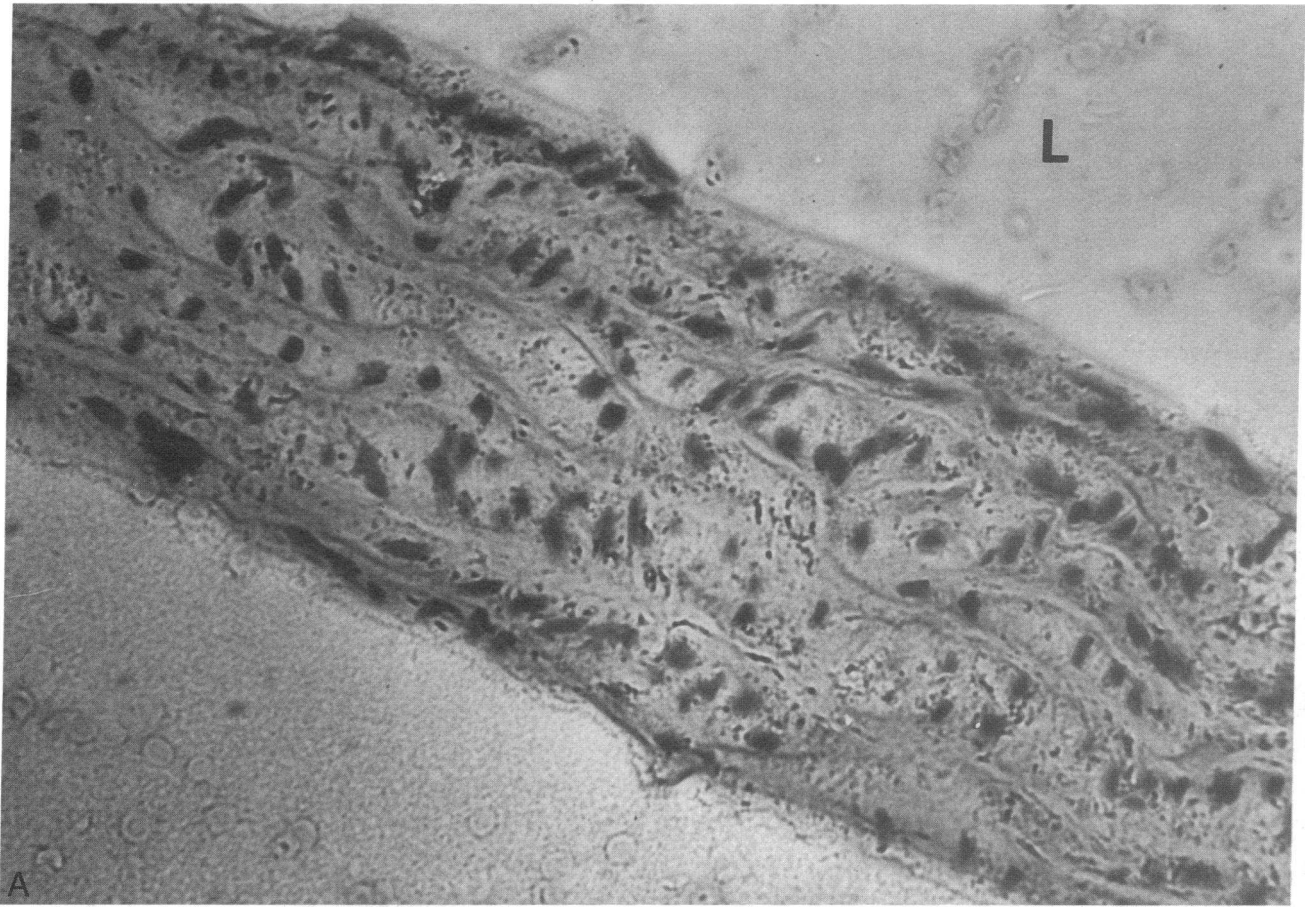
Tissue processing. 12-wk old male Wistar and WKY rats were anesthetized with ether. The animals were perfused via the left ventricle with 0.9% saline followed by 4% paraformaldehyde in 0.12 M sodium phosphate, pH 7.4. Tissue was rapidly dissected and placed in 4% paraformaldehyde in 0.12 M sodium phosphate, after which it was cleared in xylene and embedded in paraffin. 7- μm cross-sections were then cut on a rotary microtome and placed onto poly-L-lysine (0.01%)-treated glass slides.

Sections were baked onto slides at 80°C and after removing paraffin with xylene and passing through ethanol, the slides were postfixed in 4% paraformaldehyde for 20 min, dipped in $3 \times \text{PBS}$, $1 \times \text{PBS}$, $1 \times \text{PBS}$ for 5 min each. The slides were dehydrated with 30–100% ethanol and stored at 20°C until hybridized.

Prehybridization. All reagents were treated with 0.1% diethyl pyrocarbonate to prevent RNA degradation. Unless specified, all steps were performed at room temperature. The slides were dipped in 0.2 M HCl for 20 min, deionized water for 5 min, and $2 \times \text{SSC}$ for 15 min, followed by a 30-min wash in $0.1 \times \text{SSC}$ at 65°C ($1 \times \text{SSC}$ consists of 0.15 M NaCl, 0.01 M Na citrate). Following a 5-min deionized water wash, the slides were treated with 50 μl proteinase K (0.25 mg/ml proteinase K in 50 mM Tris HCl, pH 7.5, and 5 mM EDTA). They were then rinsed for 5 min in 4% paraformaldehyde, followed by PBS and PBS/glycine (2 $\mu\text{g}/\text{ml}$) washes. The slides were once more immersed in 4% paraformaldehyde (20 min), rinsed for 5 min in PBS, and treated for 10 min with 0.25% acetic anhydride in 0.1 M triethanolamine to reduce background hybridization. The slides were then rinsed in PBS, followed by successive dehydration in ethanols with 0.3 M ammonium acetate for 5 min each (50, 60, 80, 94, and 100% ethanol).

Hybridization. The slides were prewarmed for 15 min to 42°C and 500,000 cpm/slide of ^{35}S -labeled cRNAs for angiotensinogen and control probes (see below) were placed on sections in a vol of 50 μl each. Hybridization buffer consisted of 50% formamide, 30 mM NaCl, 5 mM EDTA, $1 \times \text{Denhardt's}$ (0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% BSA) and 20 mM Tris HCl. The slides were then incubated for 4–6 h at 42°C in a humidified, sealed container in which the same hybridization buffer was placed to maintain moisture.

After hybridization, the slides were washed successively in $4 \times \text{PBS}$, treated with RNase A-containing buffer (500 mM NaCl, 10 mM Tris HCl, pH 8.0) for 1 h, and rinsed in buffer alone for 30 min. The slides were then washed in 2 liters of $2 \times \text{SSC}$ twice for 30 min at 45°C and then $0.1 \times \text{SSC} \times 2$ for 45 min. They were dehydrated, air fixed, and dried. Photographic emulsion was applied in total darkness (Kodak NTB-2, diluted 1:1 in 0.6 M ammonium acetate). After the slides had been air dried for 2 h, they were placed in light-tight boxes and stored at 4°C for 5 wk. They were then developed using Kodak D-19 developer,



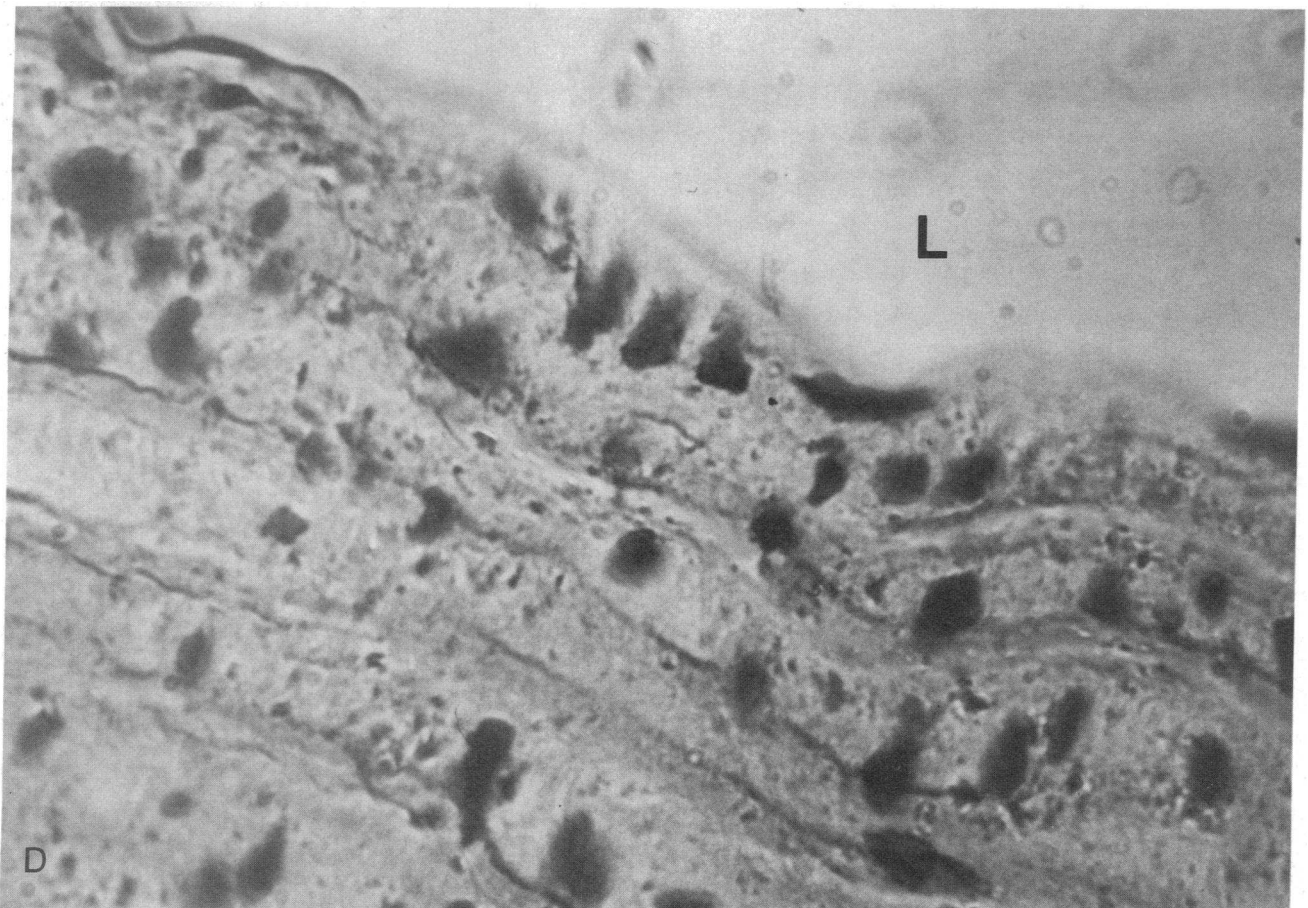
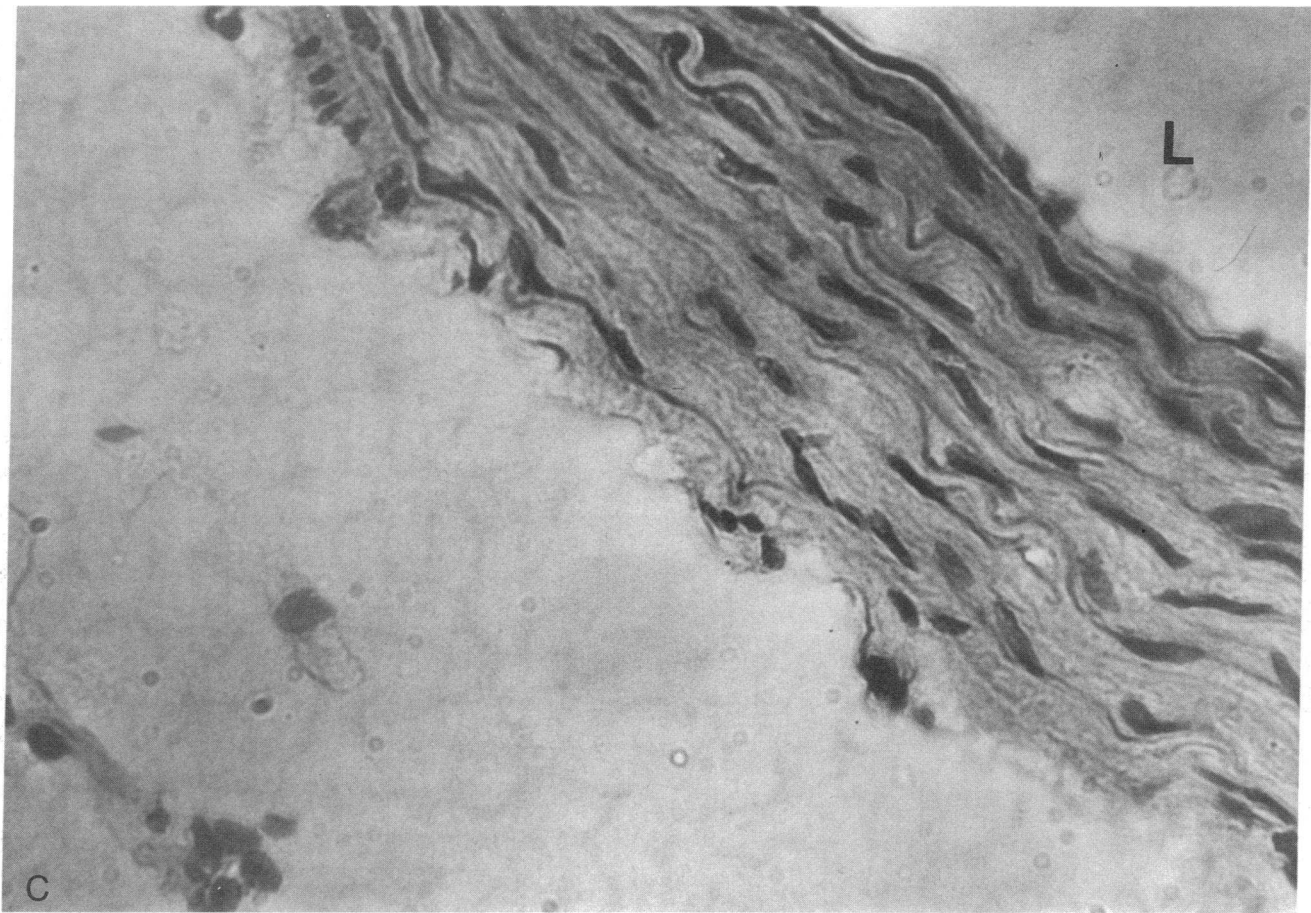


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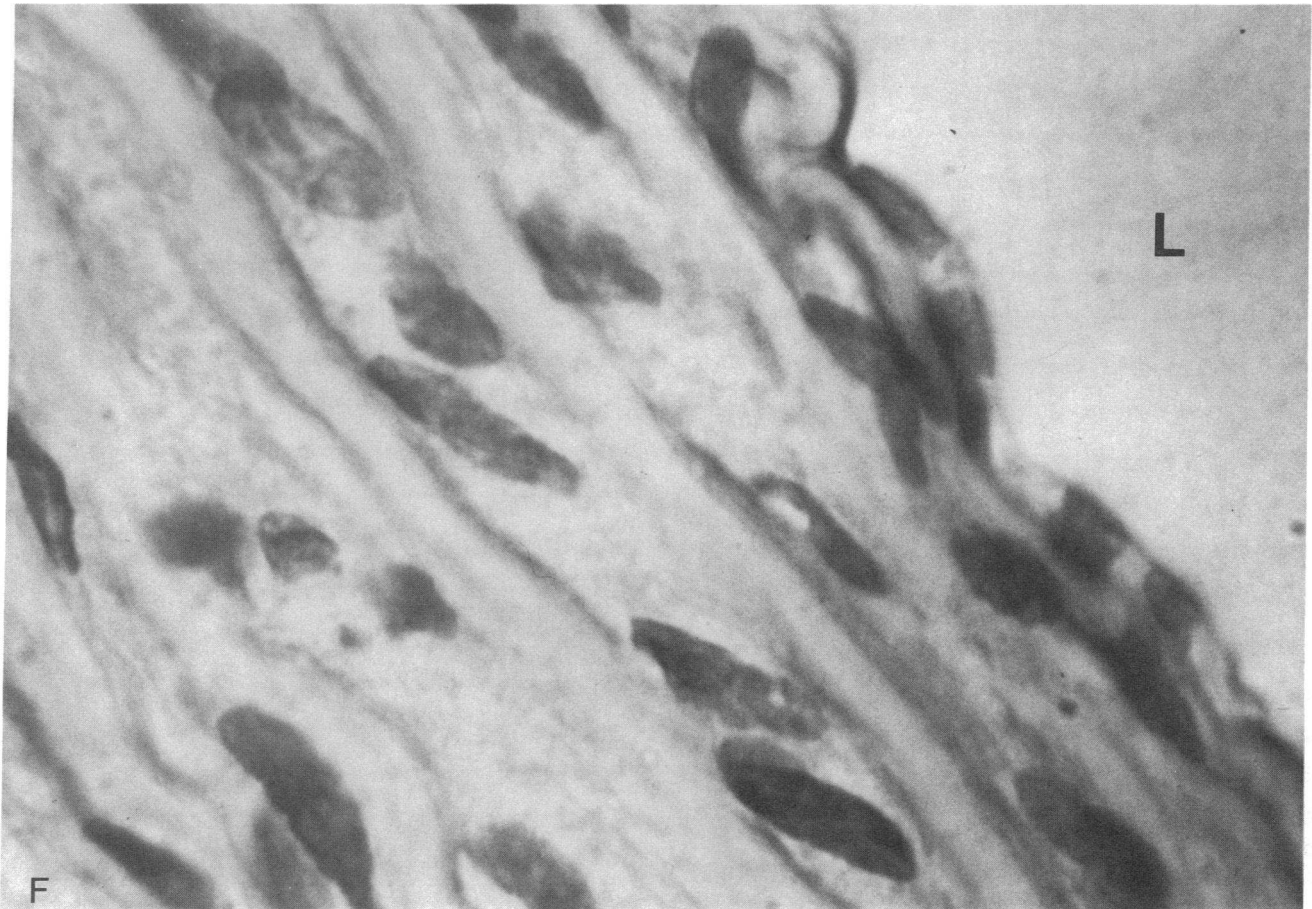
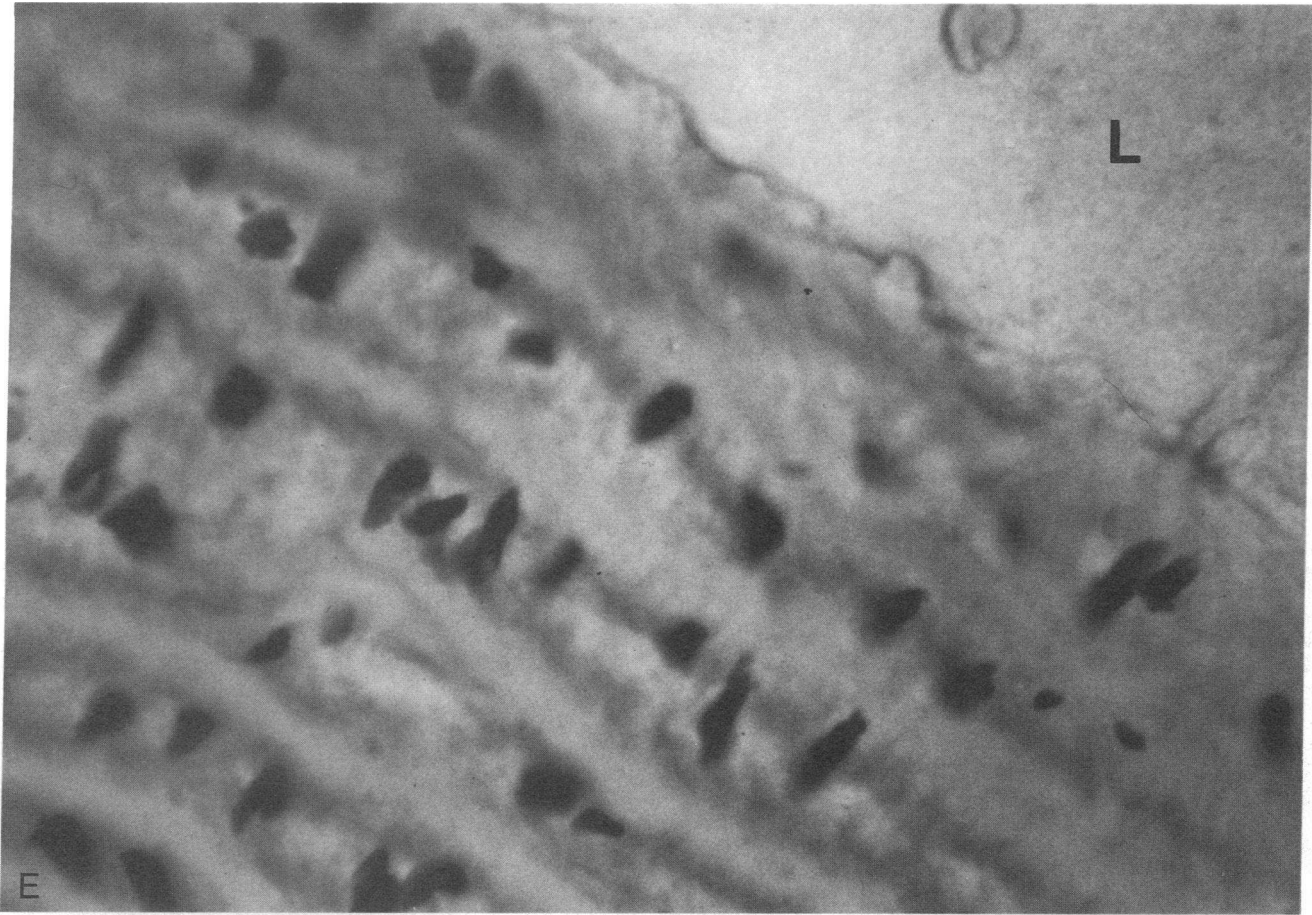


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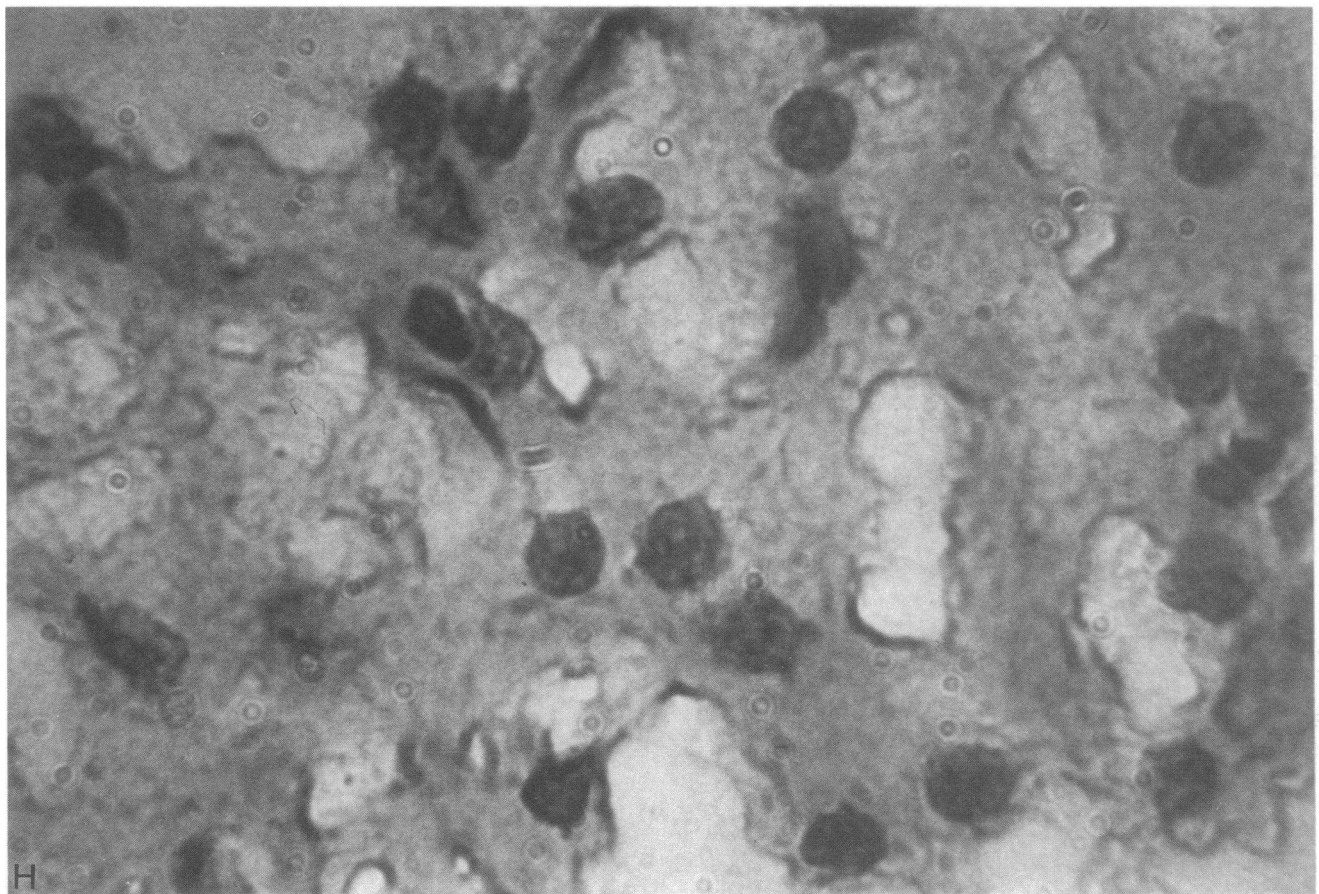
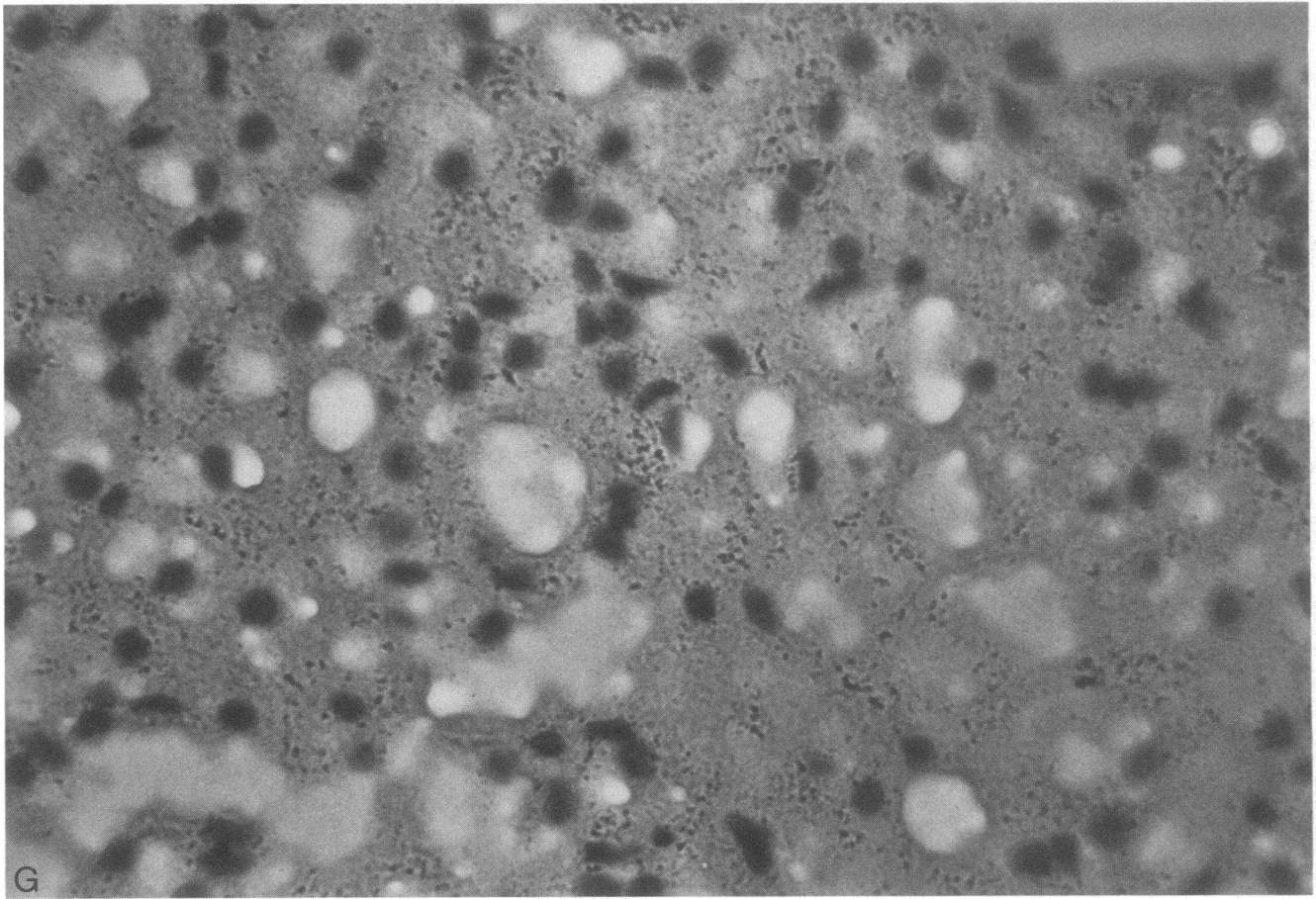


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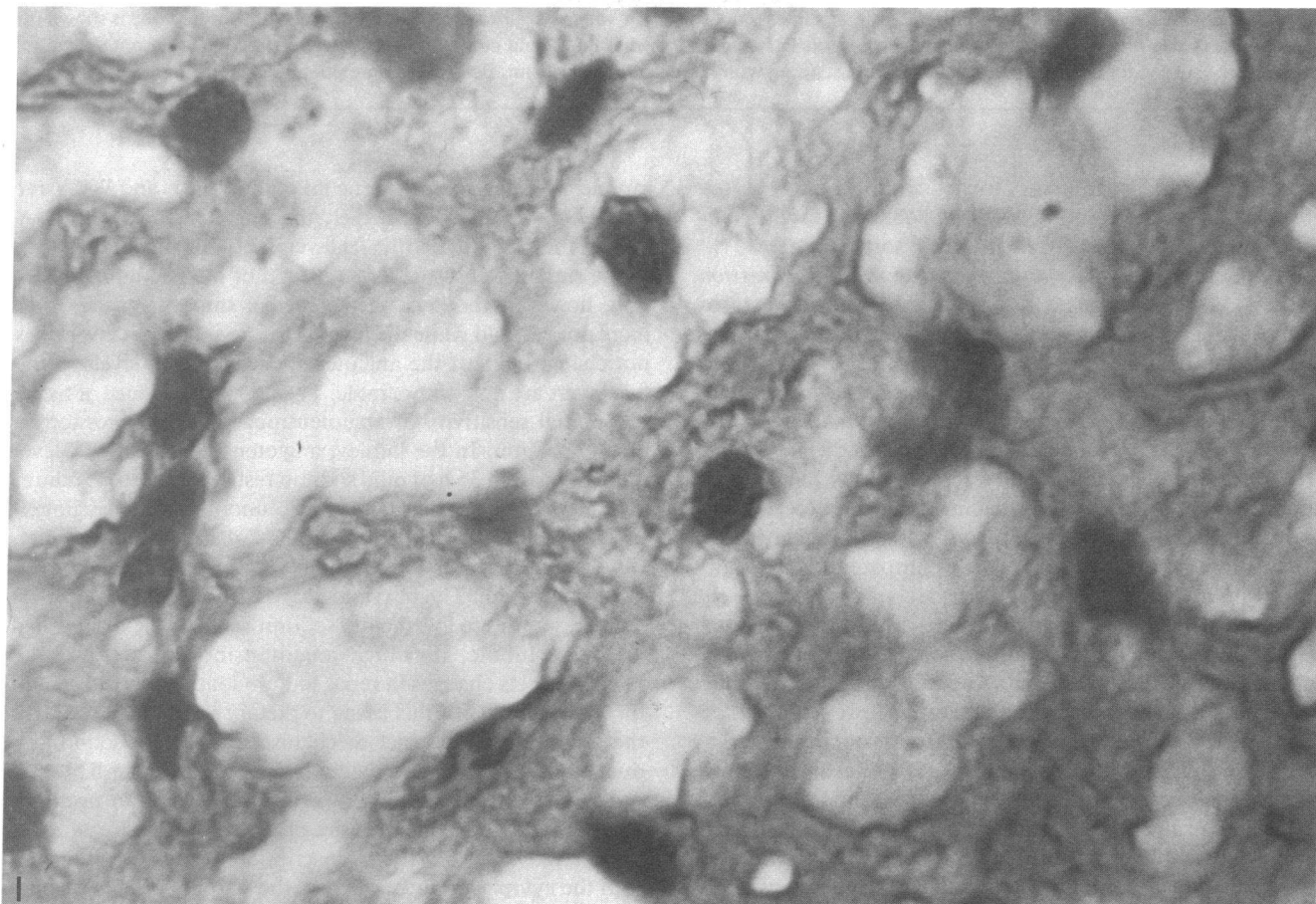


Figure 2 (Continued)

counterstained with hematoxylin and eosin, and examined with a Nikon bright-field/dark-field microscope and photographed.

Probe preparation. For in situ hybridization studies, a 700-bp fragment of pRang 1650, a full-length rat angiotensinogen cDNA (9), was cloned into Blue Scribe Vector. When linearized this could be transcribed with T₃ polymerase to generate an antisense cRNA that was labeled with 35S UTP (400 Ci/mmol). Using T7 polymerase with the same construct, we generated a sense cRNA for a negative control. Additional negative controls consisted of cold competition with cold-labeled antisense probe and pretreatment of slides with RNase A to destroy the native RNAs in the tissue sections. Positive control tissue consisted of liver and kidney.

Physiologic parameters. Blood pressures were measured in conscious unrestrained rats on the fourth day of the diet by a catheter placed in the femoral artery 48 h previously. The blood pressure for each animal was averaged over a 5-min period. Each rat was placed in a metabolic cage and daily urine outputs were collected. 24-h urinary sodium concentration was measured using a flame photometer (model 443; Instrumentation Laboratory, Inc., Lexington, MA).

Statistical analysis. Differences between groups were determined by Student's unpaired *t* test and *P* < 0.05 was considered significant.

Results

Distribution of angiotensinogen mRNA in the aorta by Northern blot analysis. RNA from aorta stripped of its endothelium and adventitia was first analyzed by Northern blotting. Angiotensinogen cDNA readily hybridizes to the RNA extracted

from this medial layer of the aorta (Fig. 1 *a*). The estimated size of aortic medial angiotensinogen mRNA is ~ 1.7 kb which is in agreement with previous reports (7, 9) and is indistinguishable in size from that of rat liver angiotensinogen mRNA. To confirm that the stripped aorta contained primarily medial smooth muscle layer, we examined the morphology of the stripped aortae that were used for the RNA studies. Fig. 1 *b* shows a representative section. The majority of the tissue was the medial layer containing smooth muscle cells, with no endothelium remaining and containing only a small amount of adventitia. No adipose tissue was detected.

Northern blot analysis of the RNA extracted from the periaortic fat also demonstrates the expression of angiotensinogen mRNA in this region (Fig. 1 *a*). Periaortic angiotensinogen mRNA is indistinguishable in size from medial smooth muscle angiotensinogen mRNA.

The same regional distributions and migration characteristics can be seen for the angiotensinogen mRNA in the Wistar aorta (data not shown).

Localization of angiotensinogen mRNA in the aorta by in situ hybridization. Angiotensinogen mRNA is present in Wistar aortic media and in periaortic fat as demonstrated in Fig. 2. Positive grains can also be observed in the endothelial cells, albeit at lower levels. In contrast, a minimal amount of silver grains (consistent with nonspecific background) is seen in the tissue incubated with the labeled sense probe, or in tissues subjected to cold competition at the time of hybridization with

Figure 3. Angiotensinogen mRNA detection in aortic medial layer of the WKY rats. Hybridization with radiolabeled antisense probe is shown in *A*; the control (sense probe) is shown in *B*. Other controls (RNase pretreatment and cold competition) were also negative (data not shown). Sections were exposed for 5 wk before developing and staining with hematoxylin and eosin. Both fields were photographed at $\times 100$.

the antisense probe or in tissues treated with RNase A before hybridization (Fig. 2). Similar results are obtained for distribution of angiotensinogen mRNA in WKY aorta (Fig. 3).

Regulation of regional angiotensinogen mRNA expression and physiological parameters by sodium diet. 5 d of low sodium diet increased angiotensinogen mRNA level (as measured by blot analysis) in the aortic media of WKY rat by 3.2-fold ($P < 0.005$, $n = 6$ pooled samples for each diet), as compared with the normal sodium diet (Fig. 4). These differences were specific for angiotensinogen and not due to variable RNA purity or degradation since reprobing the blots with a mouse β actin probe showed no significant differences between the groups (5.6 ± 0.6 arbitrary U/ μ g vs. 6.1 ± 0.2 arbitrary U/ μ g; $P = \text{NS}$, low vs. normal sodium). To compare directly angiotensinogen mRNA levels in the aortic smooth muscle and the adipose tissue, the RNA from the two regions were quantitated together. Fig. 4 shows that on a 1.6% sodium diet the angiotensinogen mRNA level in the periaortic fat was 2.6-fold higher than in the medial smooth muscle layer. However, angiotensinogen mRNA level in the periaortic fat was not influenced by sodium so that on 5 d of the 0.02% diet the levels in the two layers became comparable ($n = 4$ pooled samples for each diet).

We also examined the time course of the increase of medial smooth muscle angiotensinogen mRNA expression that was inducible by low sodium diet. No increase in angiotensinogen mRNA level was observed after 1 d of low sodium diet (the ratio of angiotensinogen mRNA levels on low/normal salt diet was 0.85; $n = 5$ for each diet, $P = \text{NS}$). By day 3 of low sodium diet, there was a tendency for medial angiotensinogen mRNA level to increase (the ratio of mRNA levels on low/normal salt diet was 1.5, $n = 5$ for each diet, $P = 0.1$). By 5 d, as demonstrated above, there was a significant increase in aortic medial angiotensinogen mRNA. As reported previously, kidney angiotensinogen mRNA level increased significantly while liver angiotensinogen mRNA did not change after 5 d of low sodium diet (8).

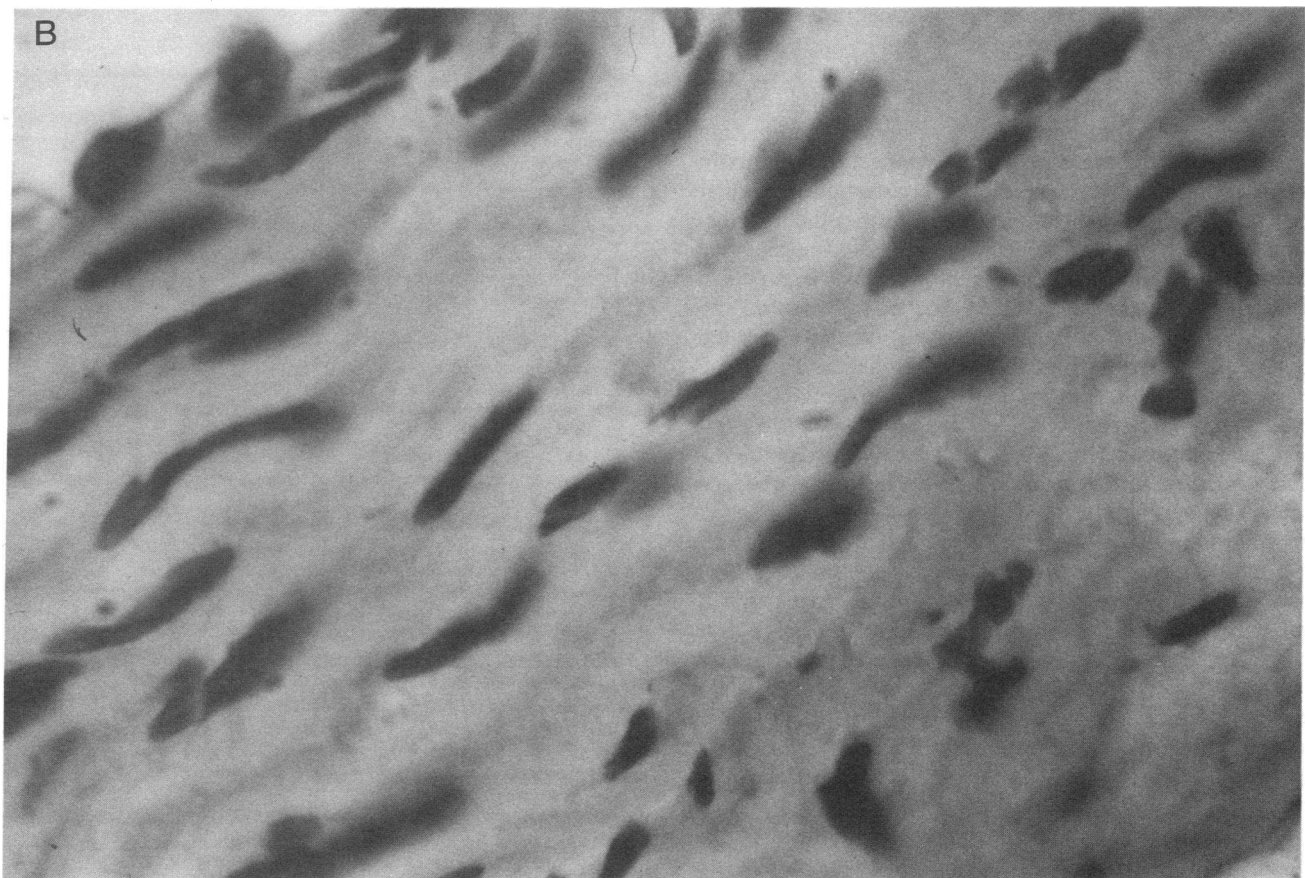
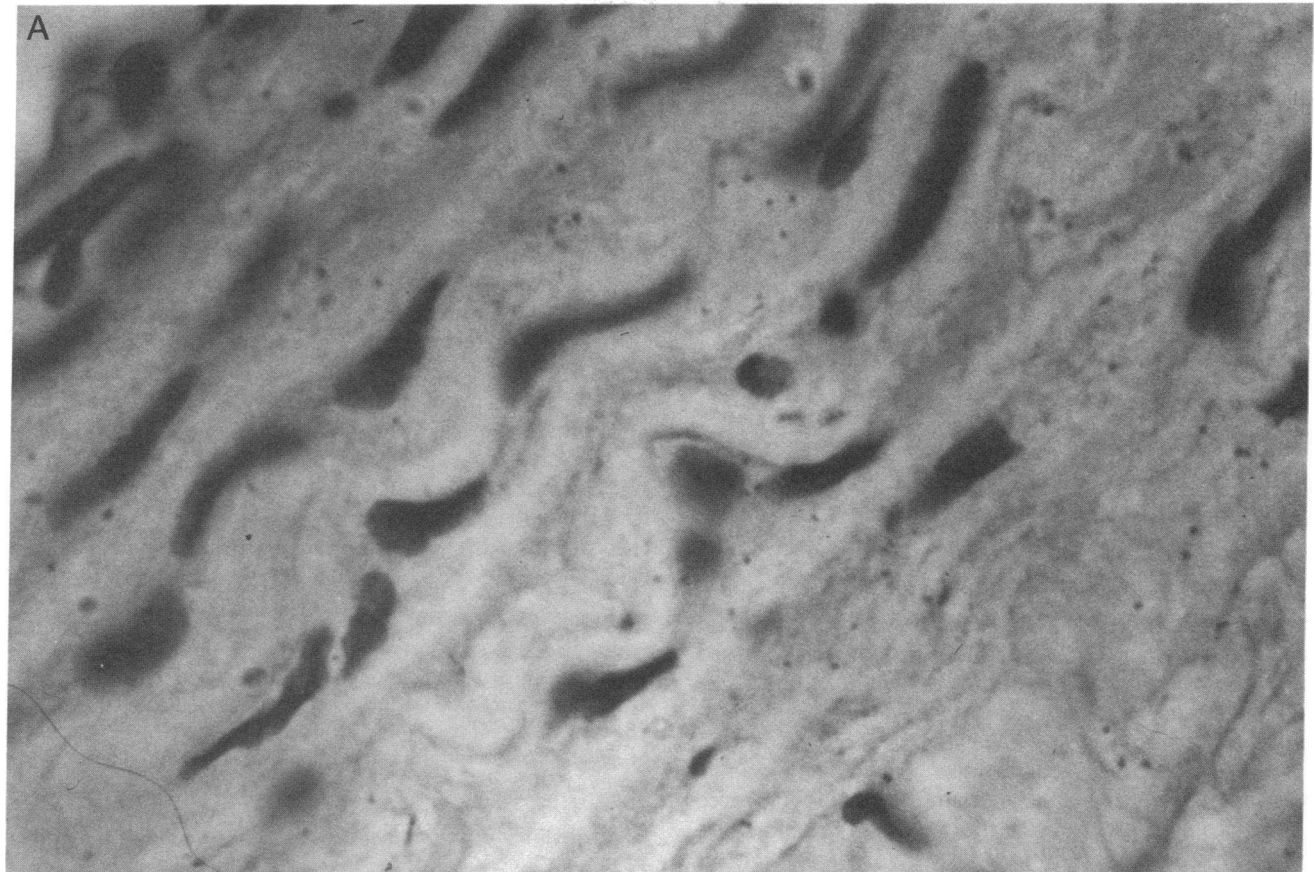
The physiological correlations of tissue angiotensinogen expressions that were induced by low sodium diet were also studied. The metabolic balance was assessed by measuring the total urinary sodium output ($U_{\text{Na}}V$) for each 24-h period. After 48 h, the animals on the 1.6% sodium diet were in balance since no further change in $U_{\text{Na}}V$ was noted (5.25 ± 0.31 meq at day 4, $P = \text{NS}$, $n = 8$). Animals on the low sodium diet took longer to come into balance since $U_{\text{Na}}V$ was still lower on day 4 than on day 2 (0.05 ± 0.01 meq vs. 0.08 ± 0.02 meq, $P < .01$, $n = 8$). On day 5, no further change in $U_{\text{Na}}V$ was noted on either diet. The sodium diets did not alter blood pressure. The direct mean arterial pressures as measured on the fourth day were not significantly different between the low and high sodium rats (122 ± 3 mmHg vs. 125 ± 3 mmHg, respectively, $n = 6$).

Discussion

Using both Northern blot analysis and in situ hybridization histochemistry, the data presented herein demonstrate that angiotensinogen mRNA is expressed in both the medial smooth

muscle layer and the periaortic fat of the Wistar and WKY rat aorta. On a 1.6% sodium diet, the angiotensinogen mRNA level in the WKY periaortic fat layer is 2.6-fold higher than that in the smooth muscle layer. With 5 d of mild sodium restriction, however, the level in the medial smooth muscle layer increases 3.2-fold while the level in the periaortic fat layer does not change, so that the angiotensinogen mRNA levels in the two layers are now comparable. There is also evidence of tissue differential sensitivity of angiotensinogen gene expression to dietary sodium. In the kidney, angiotensinogen mRNA level increases within 36 h of mild sodium restriction (0.02% sodium diet) (15). On the other hand, aortic smooth muscle angiotensinogen mRNA increases only after 5 d of low sodium diet, while periaortic fat and liver angiotensinogen mRNA levels do not change at all. The physiological basis for such tissue differential responses to low dietary sodium is not known. The renal proximal tubule, in which angiotensinogen is expressed, readily detects changes in renal sodium load and may account for the sensitivity of this tissue to dietary sodium changes. On the other hand, the exact mechanism for dietary sodium-induced changes in aortic medial angiotensinogen mRNA expression is unclear. Changes in mRNA levels can occur by altering the rate of one or more of the steps necessary for the production of mRNA (i.e., transcription, processing, transport into the cytoplasm) or by altering the stability of the cytoplasmic mRNA. More detailed experiments would need to be performed in order to clarify this point. It is interesting to note that a significant change in medial angiotensinogen mRNA level was only seen after the rats achieved sodium balance with their diets.

These data also emphasize the importance of sodium diet in affecting the distribution of angiotensinogen mRNA expression in the rat aorta. Using Northern blot analysis, Campbell and Habener (11) measured angiotensinogen mRNA in the vessel wall versus the adherent connective and brown adipose tissues of Sprague Dawley rat aorta. They observed that the vessel wall angiotensinogen mRNA signal as related to the total RNA in the tissue was one-third that in the adherent fat tissue. These results are consistent with our data in the WKY rats that show that on a normal (1.6%) sodium diet, the medial smooth muscle angiotensinogen mRNA concentration (as related to total RNA) is about one-third of that in the periaortic fat. In a study using in situ hybridization histochemistry, Campbell and Habener reported that angiotensinogen mRNA was primarily localized in the periaortic adipose tissue in Sprague Dawley rats treated with estrogen, thyroxine, and dexamethasone (11). This combination drug treatment, known to influence region-specific expression of angiotensinogen mRNA (6), resulted in a 12-fold increase in angiotensinogen mRNA level (as analyzed by Northern blot technique) in the periaortic adipose tissue with minimal change in the angiotensinogen mRNA level in the medial layer of the vessel wall (11). This marked increase in the periaortic fat angiotensinogen mRNA favored its detection in this region by in situ hybridization and might influence the overall interpretation of the autoradiographic localization experiment. Using Northern blot analysis, Cassis et al. (12) de-



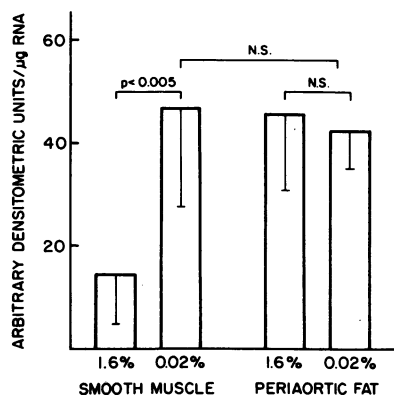


Figure 4. Relative levels of angiotensinogen mRNA in the medial smooth muscle layer and periaortic fat layers of WKY rats on 0.02 and 1.6% sodium diets. Total RNA was isolated from the indicated regions and quantitated by slot blot analysis as described in Methods. $n = 6$ for the smooth muscle layer (on each diet) and $n = 4$ for the periaortic fat layer (on

each diet). For the smooth muscle, each sample contained pooled tissue from three animals. For the periaortic fat layer, each sample contained pooled tissue from two animals. Error bars indicate standard errors.

tected angiotensinogen mRNA primarily in the adventitial fat of aortae of rats on uncontrolled diets. These investigators used trypsin to isolate the smooth muscle cells before RNA extraction. In our hands, trypsin treatment leads to degraded RNA. Our data suggest that a more careful analysis of the aortae from animals under controlled conditions would show a more widespread expression of angiotensinogen mRNA that is influenced by dietary sodium. In particular, the use of high resolution in situ hybridization histochemistry provides clear data on the expression of angiotensinogen mRNA in the medial smooth muscle, periaortic fat, and to a lesser extent in the endothelium as well.

It is not the purpose of this paper to examine all the components of the renin angiotensin system. Previous data suggest that vascular renin may be the result of uptake from plasma (16), whereas angiotensin converting enzyme and angiotensin receptor are locally synthesized. Our demonstration of vascular angiotensinogen gene expression support further the hypothesis of a local vascular angiotensin system. We have postulated previously that the local production of angiotensin in the blood vessel wall may exert an autocrine-paracrine influence on vascular function. Smooth muscle-derived angiotensin may influence vascular function by an autocrine mechanism. Local angiotensin may also act on its prejunctional receptors on the noradrenergic nerve endings facilitating catecholamine release by a paracrine mechanism (17). It has been proposed that intracellular angiotensin II may have a direct intracrine effect on vascular myocyte function since specific saturable angiotensin II receptors have been detected in cellular nuclei (18) and a direct effect of angiotensin II on gene transcription has been reported (19). Since angiotensin II has recently been shown to stimulate growth of cultured smooth muscle cells as well as increase the expression of several protooncogenes and autocrine growth factors (20–23), it may be postulated that this locally produced angiotensin II may be responsible, at least in part, for induction of genes responsible for smooth muscle cell hypertrophy or hyperplasia. Indeed, Powell et al. reported recently that angiotensin converting enzyme inhibition attenuated the myointimal proliferative response induced by balloon injury in vivo (24). The role of periaortic fat angiotensino-

gen gene expression is not known. Since sympathetic nerve endings traverse and terminate in this and adjacent areas, one may speculate that angiotensin derived from periaortic fat may influence sympathetic function and, hence, regulate vascular tone and growth. Further studies demonstrating the production of angiotensin in these regions of the blood vessel are of obvious importance.

What is the physiological relevance of the differential sodium responsiveness in angiotensinogen expression between these two regions of the blood vessel wall? It is reasonable to speculate that an increase in smooth muscle angiotensinogen expression induced by sodium restriction increases local angiotensin production and hence vascular tone. The significance of the lack of sodium regulation of periaortic fat angiotensinogen expression is unclear. This observation raises the possibility that angiotensinogen in fat may not be directly involved in the acute regulation of vascular tone but may exert a more long-term influence. Furthermore, it may have important nonvascular functions, such as thermogenesis and metabolism.

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