Renal Nerves Modulate Renin Gene Expression in the Developing Rat Kidney with Ureteral Obstruction

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Abstract

Chronic unilateral ureteral obstruction (UUO) in newborn rats activates renin gene expression in the obstructed kidney, and increases renin distribution along afferent glomerular arterioles in both kidneys. To investigate the role of the renal nerves in this response, 2-d-old Sprague-Dawley rats were subjected to UUO or sham operation. Chemical sympathectomy was performed by injection of guanethidine, whereas, control groups received saline vehicle. At 4–5 wk, renal renin distribution was determined by immunocytochemistry, and renin mRNA levels were determined by Northern blot hybridization. Compared to the saline-treated rats with UUO, renin remained localized to the juxtaglomerular region in both kidneys of rats with UUO receiving guanethidine ($P < 0.05$). Moreover, renin mRNA levels were eightfold lower in obstructed kidneys of rats receiving guanethidine than in those receiving saline. Additional groups of rats with UUO were subjected to unilateral mechanical renal denervation: renin gene expression in the obstructed kidney was suppressed by ipsilateral but not by contralateral renal denervation. These findings indicate that either chemical or mechanical denervation suppressed the increase in renin gene expression of the neonatal kidney with ipsilateral UUO. We conclude that the renal sympathetic nerves modulate renin gene expression in the developing kidney with chronic UUO. (*J. Clin. Invest.* 1991. 87:800–810.) Key words: renal nerves • guanethidine • sympathectomy • renin • messenger RNA • immunocytochemistry • ureteral obstruction • newborn

Introduction

There are close links between renal noradrenergic neurons and juxtaglomerular (JG)[1] cells which synthetize, store, and release renin. Anatomically, the sympathetic nerve terminals are found in close association with the cells of the afferent arteriole and the granular JG cell (1, 2). On a functional basis, renal sympathetic nerve activity modulates renin release independent of the baroreceptor and macula densa mechanisms, presumably by an action mediated by B_1 adrenergic receptors located on JG cells (3). What is not known, however, is the role of the sympathetic nerves in the modulation of renin gene expression. Of particular interest is the contribution of the renal nerves in the modulation of renin gene expression in physiologic or pathophysiologic states characterized by enhanced intrarenal renin production. Of equal importance is the interaction between the renal nerves and the intrarenal renin-angiotension system (RAS) at the level of gene expression during early development, a time in which intrarenal renin expression and synthesis are markedly enhanced (4, 5), whereas functional innervation of the kidneys is incomplete (6). Physiologic studies indicate that the intrarenal RAS is activated in animals subjected to chronic unilateral ureteral obstruction (UUO) (7–12). In contrast to the adult rat kidney, in which renin gene expression is not activated by chronic UUO (13), 1-mo-old rats subjected to UUO since day 2 of life manifest a significant increase in renin content and in the percent of JG apparatuses containing renin and its mRNA in the obstructed kidney as compared with sham-operated controls (14). Interestingly, the distribution of immunoreactive renin also was found to be increased in the intact opposite kidney, suggesting communication between the two kidneys (14). The factors that modulate renin gene expression in response to UUO and the mechanisms underlying the interactions between the two kidneys have not been investigated previously.

The present study was designed to investigate the role of the sympathetic renal nerves in modulating renin gene expression in response to chronic UUO in the newborn rat. We reasoned that given the close anatomical and functional relationships between the renal nerves and renin-containing JG cells, activation of renin gene expression in chronically obstructed kidneys could be modulated by the renal nerves.

To test this hypothesis, newborn rats subjected to UUO or sham-operation were treated chronically with guanethidine or saline injections. At 1 mo of age, the intrarenal distribution of immunoreactive renin and renin mRNA levels were assessed and the effects of sympathetic denervation (guanethidine) or vehicle (saline) administration were compared among the different groups. In addition, to assess whether the effects of guanethidine on renin gene expression were the result of renal denervation and not due to other systemic or local effects of the drug, kidney renin mRNA levels were examined in similarly obstructed rats subjected to mechanical or sham denervation of either the obstructed or intact opposite kidneys.

Portions of this study were published in abstract form (1990. *Kidney Int.* 37:354A; 1990. *Pediatr. Res.* 27:328A).

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Received for publication 5 February 1990 and in revised form 11 October 1990.

1. *Abbreviations used in this paper:* AA, afferent arteriolar segment; JG, juxtaglomerular; MAP, mean arterial pressure; RAS, renin-angiotensin system; UUO, unilateral ureteral obstruction.

J. Clin. Invest.

0021-9738/91/03/0800/11 $2.00
Volume 87, March 1991, 800–810

Methods

Protocol No. 1: systemic sympathectomy with guanethidine. These experiments were performed on 59 2-d-old Sprague-Dawley rats which were divided into four groups. Two groups were subjected to sham operation and received saline or guanethidine (Sham + S, $n = 13$; and Sham + G, $n = 12$; respectively). Two additional groups were subjected to left complete ureteral obstruction and received saline or guanethidine (UUO + S, $n = 12$; and UUO + G, $n = 22$; respectively). Under halothane and oxygen anesthesia, a small incision was made in the left lower quadrant of the abdomen and the distal ureter was ligated (UUO) or left undisturbed (Sham) (14). The abdominal wall then was closed in one layer and the animal was allowed to recover from anesthesia. Subsequently, the animals were returned to their mothers until weaning at 21 d of age, after which the rats were fed regular rat chow (Purina 5012, Ralston-Purina, St. Louis, MO) and were allowed free access to drinking water.

On day 7 of life (5 d after UUO or sham operation), guanethidine or saline injections were begun. Guanethidine monosulfate (CIBA-GEIGY Pharmaceuticals, Summit, NJ) was diluted in 0.9% saline (5–10 μl/g body weight) and was given in a dose of 50 mg/kg per d, 5 d a week for 3 wk by subcutaneous injections (a total of 15 injections) according to the method of Johnson et al. (15). Equal amounts of 0.9% saline were administered in a similar fashion to saline-treated animals.

Protocol No. 2: mechanical renal denervation. 15 rats were used for these experiments. The rat pups were subjected to left UUO at day 2 of life as described above. At 25–27 d of age, under pentobarbital anesthesia, left renal denervation ($n = 5$), right renal denervation ($n = 6$), or sham renal denervation ($n = 4$) were performed. Renal denervation was accomplished as described previously (16, 17). Under microscopic visualization, the renal artery was carefully stripped of its adventitia and subsequently coated with a solution of 10% phenol in absolute alcohol. During this procedure, the kidney and surrounding tissues were carefully protected from exposure to phenol. Sham renal denervation was accomplished by left or right abdominal incisions, visualization of the kidneys, but the renal pedicles were left undisturbed. It has been shown that this denervation procedure results in nearly total elimination of renal tissue catecholamines within 72 h (17).

At 4–5 wk of age, after an overnight fast with free access to water, the rats in protocols 1 and 2 were anesthetized with intraperitoneal pentobarbital sodium (Abbott Laboratories, Chicago, IL) (4 mg/100 g bw) and placed on a thermostatically-controlled heating table to maintain rectal temperature at 37±0.5°C. The right carotid artery was cannulated with polyethylene tubing (PE 50, Clay Adams, Parsippany, NJ) and mean arterial pressure (MAP) was recorded continuously by means of a Statham 23 ID pressure transducer (Gould Inc., Oxnard, CA) coupled to a Hewlett-Packard 7754 B recorder. The left ureteral diameter was measured with calipers midway between the renal pelvis and the bladder. The rats were sacrificed with saturated potassium chloride solution injected through the carotid catheter. The kidneys were removed, decapsulated, weighed, and processed for immunocytochemistry and mRNA analysis as described below.

Renin, beta-tubulin, and tyrosine hydroxylase immunocytochemistry. Seven Sham + S, 5 Sham + G, 5 UUO + S, and 6 UUO + G rats were studied. Immunocytochemical localization of renin, beta-tubulin, and tyrosine hydroxylase was performed using the ABC immunoperoxidase technique (4, 5, 13, 14). The primary antibodies used in the present study included (*a*) a specific polyclonal antirat renin antibody raised in the rabbit which binds to both renin and prorenin (diluted 1:1,000, gift of Dr. T. Inagami, Department of Biochemistry, Vanderbilt University) (18, 19), (*b*) a specific monoclonal antibody against class III beta-tubulin isotype (neuron-associated), diluted 1:200 (gift of Dr. A. Frankfurter, University of Virginia Health Sciences Center) (20), and (*c*) a specific polyclonal antibody for tyrosine hydroxylase, the enzyme responsible for the conversion of tyrosine to phenylalanine in the catecholamine synthetic pathway (Eugene Tech International, Inc., Allendale, NJ, dilution 1:4,000). After adding the secondary antibody (biotin-conjugated anti-rabbit IgG raised in the goat), the sections were incubated with avidin horseradish peroxidase complex (Vectastain ABC Kits, Vector Laboratories, Inc., Burlingame, CA), exposed to 0.1% diaminobenzidine tetrahydrochloride and 0.02% H_2O_2 as a source of peroxidase substrate, and counterstained with hematoxylin. Negative controls included omission of the primary antibody, replacement of primary antibody by nonimmune rabbit serum, and omission of secondary antibody, and no staining occurred.

Three sections per kidney were immunostained for renin and examined by light microscopy for quantitative analysis of intrarenal renin distribution. The number of renin-stained and total number of juxtaglomerular apparatuses (JGA) were counted in each section. With the aid of an ocular micrometer, the total length of the afferent arteriolar segments (AA) and the length of renin immunostaining along each AA were measured. All measurements were done on coded slides such that the investigator was unaware of the treatment groups. Because the total number of JGA varied among sections, the following immunohistochemical ratios were determined and compared among groups (5, 13, 14): %JGA = (number of stained JGA/total number of JGA) × 100; %LAA = (length of stained AA/total length of AA) × 100. The ratios thus obtained from each slide were pooled and averaged for each kidney. Comparisons were made using the final ratios obtained for each kidney. Only JGAs with a vas afferens entering the glomerulus were used for computation.

One to two sections of each kidney were stained for beta-tubulin and for tyrosine hydroxylase as a means of documenting the success of renal denervation.

Renin mRNA analysis. Total RNA from the left and right kidneys of six rats in each group in protocol 1 and from the three groups in protocol 2 was extracted according to the method of Chirgwin et al. (21) and pooled for each group. 15 μg of precipitated RNA was separated by electrophoresis on a 1.2% agarose-formaldehyde gel and then transferred from the gel to a nylon membrane (Zetabind, Cuno, Inc., Meriden, CT) by capillary action (Northern blot). To control for the different efficiencies at which mRNA species might be extracted relative to total RNA, each RNA sample was analyzed both for renin mRNA and for the mRNA of the constitutively expressed cytoskeletal protein, beta actin, as an internal standard.

The Northern blots were hybridized to a full-length renin cDNA, and to beta-actin cDNA (gifts of Dr. K. R. Lynch and Dr. G. Owens, University of Virginia) labeled with phosphorus-32 by a random primer technique (22) or by nick translation (23) to a specific activity of $> 1.6 \times 10^8$ cpm/μg, isolated using Sephadex G-50 columns (Pharmacia Fine Chemicals, Piscataway, NJ) and eluted by centrifugation. ^{32}P-cDNAs were detected by contact autoradiography and quantified by densitometry.

Renal norepinephrine content. Kidney norepinephrine contents were assayed by HPLC with electrochemical detection (Bioanalytical Systems Inc., West Lafayette, IN).

Plasma renin activity was measured by radioimmunoassay (24).

Statistical analysis. Comparisons among the groups were performed by one-way analysis of variance followed by Newman-Keuls test; and between the left and right kidneys in each group by paired *t* test. Statistical significance is defined as $P < 0.05$, and the results are presented as means±SEM.

Results

Animal survival

In protocol 1, eight rats died during the study resulting in an overall survival rate of 87%. For individual groups, the survival rate was 100% in Sham + S, 92% in Sham + G, 92% in UUO + S, and 73% in UUO + G. Guanethidine or UUO alone did not appear to cause a significant mortality in the growing newborn

Table I. Characteristics of Animals in the Chemical Sympathectomy Protocol

Group	Body weight	LKW/100 g body weight	RKW/100 g body weight	Left ureteral diameter	MAP
	g	*g*	*g*	*mm*	*mmHg*
SHAM + S (n = 10)	85.0±5.2	0.50±0.01	0.52±0.01	1.00±0.03	97.0±6.5
SHAM + G (n = 11)	76.3±1.5	0.47±0.01	0.49±0.01	0.90±0.02	92.0±2.6
UUO + S (n = 7)	78.0±4.0	0.33±0.06[¶‖]	0.77±0.02[‖]	2.20±0.20[‡§]	101.0±3.1
UUO + G (n = 16)	70.0±2.5*	0.45±0.02[¶]	0.67±0.02[‖]	1.80±0.10[‡]	82.0±5.0

LKW, left kidney weight; RKW right kidney weight. Mean arterial pressure (MAP) was measured in 7 Sham + S, 10 Sham + G, 4 UUO + S, and 11 UUO + G rats. * $P < 0.05$ vs. Sham + S; [‡] $P < 0.01$ vs. Sham + S and Sham + G; [§] $P < 0.05$ vs. UUO + G; [‖] $P < 0.01$ vs. other groups; [¶] $P < 0.01$ vs. right kidney; mean±SEM.

rats, whereas the combination of ureteral obstruction and systemic sympathectomy was associated with increased mortality. In protocol 2, survival rate was 100%.

Somatic and renal growth

Compared to saline-treated rats, chemical sympathectomy with chronic guanethidine treatment did not affect body weight in sham-operated rats. Similarly, saline-treated animals subjected to chronic neonatal UUO did not suffer from any growth deficit. However, compared to the Sham + S group only, UUO rats with sympathectomy (group UUO + G) had a significantly lower body weight by the end of the third week of guanethidine treatment (Table I). Body weights were not different among the three groups subjected to sham or mechanical denervation (Table II).

The effects of UUO on renal growth were different in the saline-treated and the sympathectomized animals (Table I). Compared with sham-operated kidneys, a significant growth arrest occurred in the obstructed kidneys of UUO + S but not UUO + G rats. In addition, the left ureteral dilation was significantly greater in saline-treated than in sympathectomized rats ($P < 0.05$) (Table I). Compensatory hypertrophy occurred in the intact opposite kidneys of both UUO + S and UUO + G animals. However, the hypertrophic response was significantly greater in the intact kidneys of UUO + S than of UUO + G groups (Table I).

Unlike sympathectomy, neither ipsilateral nor contralateral renal denervation had an effect on the degree of growth arrest in the obstructed kidneys as compared with sham-mechanical denervation rats. The magnitude of compensatory hypertrophy in the intact kidney with contralateral UUO was similar in the three mechanical denervation groups (Table II).

Mean arterial pressure

There were no statistically significant differences among the groups in MAP, although the UUO + G rats tended to have slightly lower blood pressures (Table I). Similarly, MAP was similar in all three mechanical denervation groups (Table II).

Kidney norepinephrine content, beta-tubulin and tyrosine hydroxylase immunoreactivity

Guanethidine administration resulted in an 80–90% decrease in renal norepinephrine content as compared to the values in saline-treated rats (Table III). As shown in Table IV, mechanical denervation resulted in a significant suppression of renal norepinephrine content in both UUO and sham groups. In saline-treated rats of either sham or UUO groups, beta-tubulin and tyrosine hydroxylase immunoreactivity was distributed mainly along the adventitia of the major intrarenal vessels (arcuate, interlobular) with occasional staining along the afferent

Table II. Characteristics of Animals in the Mechanical Denervation Protocol

Group	Body weight	LKW	RKW	MAP
	g	*g*	*g*	*mmHg*
UUO + LMD (n = 5)	64.0±9.1	0.14±0.02*	0.59±0.08	123.0±11.0
UUO + RMD (n = 6)	74.0±6.3	0.23±0.05*	0.64±0.03	124.0±4.4
UUO + SMD (n = 4)	80.0±5.1	0.19±0.05*	0.68±0.07	115.0±9.0

LMD, left mechanical denervation. RMD, right mechanical denervation. SMD, sham-mechanical denervation. * $P < 0.05$ vs. right kidney. Mean±SEM.

Table III. Guanethidine denervation kidney norepinephrine content

Group	Left kidney	Right kidney
	μg/g kidney wt	*μg/g kidney wt*
Sham + S	0.240±0.030	ND
Sham + G	0.030±0.010*	ND
UUO + S	0.120±0.020[‡]	0.130±0.010
UUO + G	0.019±0.003*	0.017±0.004*

* $P < 0.01$ vs. the corresponding saline-treated group; [‡] $P < 0.05$ vs. sham + S. ND, not done. n = 5 in each group. Mean±SEM.

Table IV. Mechanical denervation kidney norepinephrine content

Group	Denervated kidney	Nondenervated kidney
	μg/g kidney wt	*μg/g kidney wt*
UUO + LMD	0.071	0.118
UUO + RMD	0.001	0.142
SHAM + LMD	0.004	0.150
SHAM + RMD	0.032	0.137
Mean*	0.027	0.137
SEM	0.016	0.007

* $P < 0.02$.

arterioles (Fig. 1, *A* and *B*). Beta-tubulin and tyrosine hydroxylase immunoreactivity was not detected in the kidneys of guanethidine-treated animals of either sham or UUO groups.

Intrarenal renin distribution

There were no statistically significant differences in the number (mean±SEM) of total JGAs per rat used for computations among the kidneys of the experimental groups (Sham + S: left kidney 66±15.9, right kidney 70±8; Sham + G: left kidney 55±7.7, right kidney 61±5.7; UUO + S: left kidney 61±4.7, right kidney 68±9; UUO + G: left kidney 74±22.7, right kidney 59±10.8).

Effect of sympathectomy in sham-operated rats. No differences were noted in the distribution of immunoreactive renin in the kidneys of saline-treated (Sham + S) versus guanethidine-treated (Sham + G) rats. In both groups, renin was confined to those cells of the afferent arterioles that were juxtaglomerular in location. Renin immunoreactivity was not observed beyond 45–50 μm upstream from the glomerulus along the AA. In addition, the percentage of renin-containing JGA (%JGA) and the percentage length of the AA containing renin (%LAA) were similar in these two groups of rats (Figs. 2 and 3).

Effect of UUO in saline-treated rats. In contrast to sham-operated kidneys in which renin was confined to a juxtaglomerular position, renin was found along most of the identified length of the AA in the obstructed kidneys of saline-treated UUO rats (UUO + S) (Fig. 4 *A*). Although less dramatic, increased distribution of renin was seen also in the intact opposite kidneys of UUO + S rats (Fig. 4 *B*). In addition, the %JGA and %LAA were significantly higher in both the obstructed and intact opposite kidneys of UUO + S group than in those of sham-operated rats (Figs. 2 and 3).

Effect of UUO in sympathectomized rats. Renin immunoreactivity of the AA was greatly diminished in both the obstructed and intact opposite kidneys of UUO + G rats when compared to that of UUO + S kidneys (Fig. 4, *C* and *D*). Thus, compared to sham-operated kidneys, renin distribution (both %JGA and %LAA) was increased in the obstructed kidneys of UUO + S but not UUO + G rats (Figs. 2 and 3). Sympathectomy also prevented the increase in the distribution of immunoreactive renin in the intact kidneys with contralateral UUO which was seen in saline-treated UUO rats (Figs. 2 and 3).

No significant differences in plasma renin activity were found between UUO + S and UUO + G rats (mean±SEM; UUO + S [$n = 4$]: 5.7±1.8 ng/ml per h; UUO + G [$n = 4$]: 5.8±1.1 ng/ml per h).

Renin mRNA studies

As shown in the Northern blot in Fig. 5, guanethidine given chronically to sham-operated maturing rats did not appear to have an appreciable effect on renin mRNA levels relative to those of saline-treated rats. Renin mRNA levels quantitated by densitometry were eightfold higher in the obstructed kidneys of UUO + S rats than in the intact opposite kidneys and the kidneys of the remaining groups. Fig. 6 shows the results of hybridization of the Northern blot with beta actin cDNA.

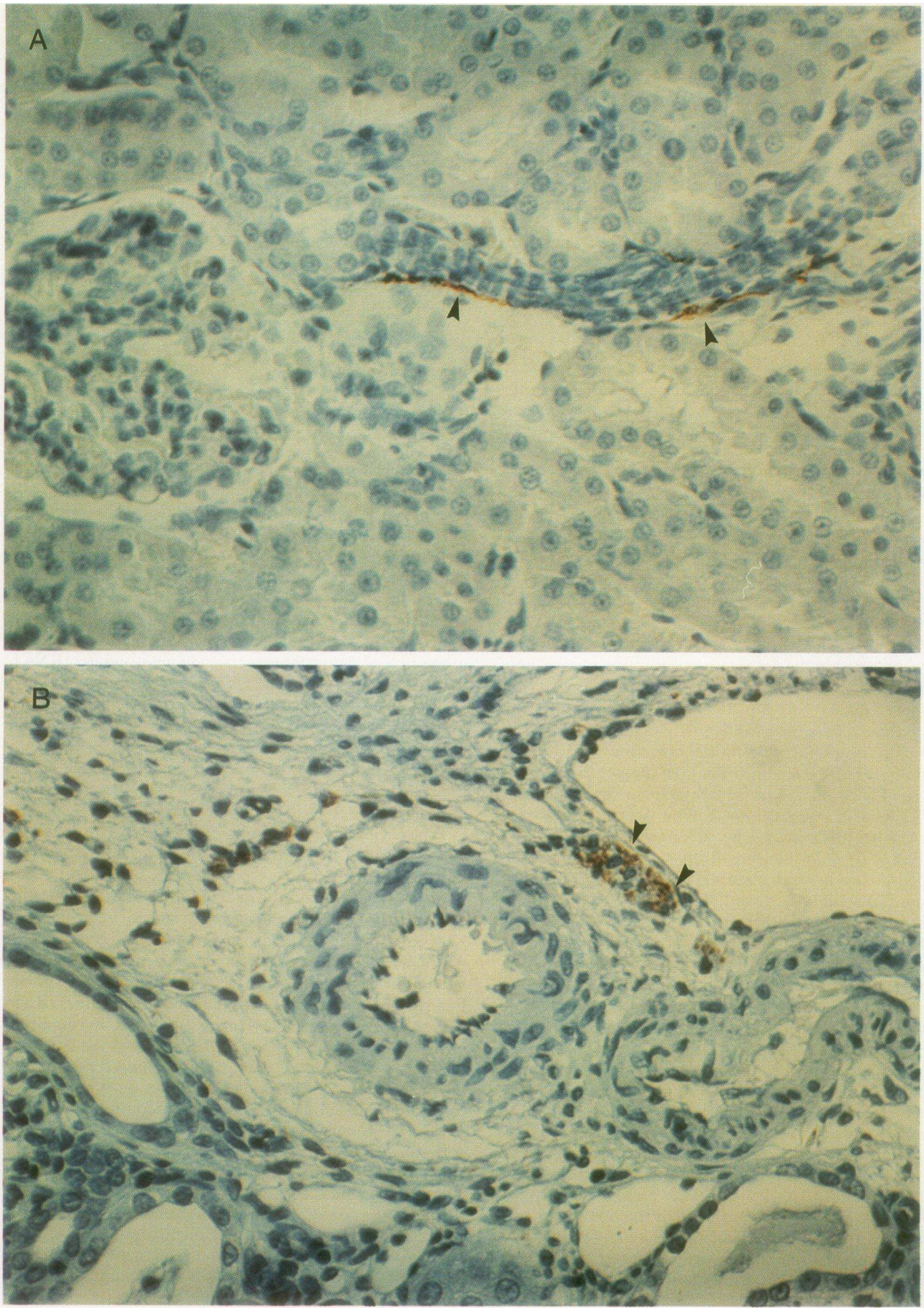
Mechanical denervation of the obstructed kidneys was associated with a fivefold decrease of renin mRNA in these kidneys, whereas contralateral denervation of the intact kidney did not affect renin gene expression in either kidney as compared to sham denervated obstructed rats (Fig. 7).

Discussion

To our knowledge the interrelationships between the sympathetic nervous system and the renin-angiotensin system at the level of gene expression have not been reported previously in the developing animal. In this respect, two main questions arise: (*a*) Are renal nerves important in the developmental regulation of renin gene expression? (*b*) What role do renal nerves play in the modulation of renin gene expression in the setting of enhanced intrarenal renin production? The purpose of the present study was to investigate the importance of the renal nerves in modulating the expression of renin and its mRNA in the developing kidney with chronic ureteral obstruction.

The results of the present study indicate that renin gene expression is activated in chronically obstructed kidneys of maturing rats and that an intact sympathetic renal innervation is required for that activation to occur. In contrast, sympathectomy did not influence the distribution of renin or the amount of its mRNA in the kidneys of nonobstructed rats.

The chemical denervation protocol employed in the present study was adapted from that of Johnson et al. (15). These investigators demonstrated that chemical sympathectomy of newborn rats with chronic guanethidine administration reduces peripheral tissue norepinephrine content by > 90%, abolishes ganglionic tyrosine hydroxylase activity, and results in destruction of peripheral noradrenergic neurons, producing a permanent sympathectomy. The present study confirms the findings of Johnson et al. Guanethidine treatment markedly reduced kidney norepinephrine content as well as completely abolished intrarenal tyrosine hydroxylase and beta-tubulin immunoreactivity (15). We adapted this protocol for the following reasons: First, the experiments of Johnson et al. were performed on rats during the newborn period, the same period during which our experiments were performed. Second, we performed UUO at day 2 of life when mechanical denervation would be technically impractical due to the small animal size. Third, this method does not affect central autonomic relay centers (15), permitting the transmission of impulses between any remaining intact afferent and efferent limbs. However, to be certain that the changes in intrarenal renin induced by chemical sympathectomy are the results of elimination of renal nerves and not due to other drug effects, we also performed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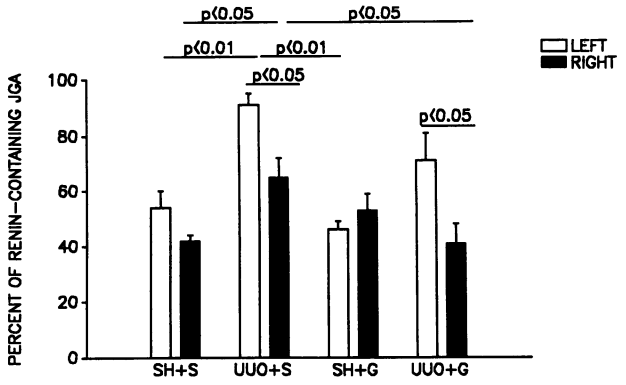


Figure 2. Percent of renin-containing juxtaglomerular apparatuses in the kidneys of the different treatment groups. Sham + S (n = 7), Sham + G (n = 5), UUO + S (n = 5), UUO + G (n = 6).

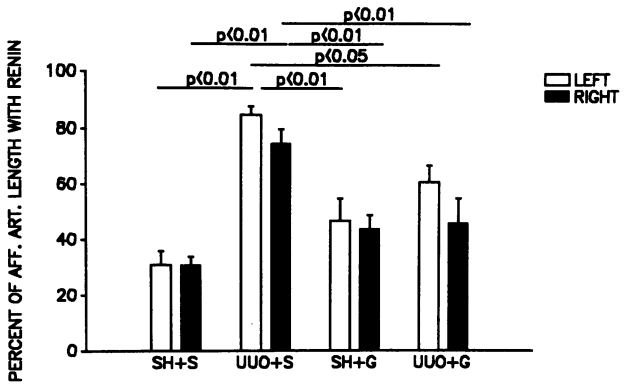


Figure 3. Percent of distal identified afferent arteriolar length with renin immunostaining. Sham + S (n = 7), Sham + G (n = 5), UUO + S (n = 5), UUO + G (n = 6).

studies in which ipsilateral or contralateral mechanical renal denervation were accomplished. Due to the small size of the animals, mechanical denervation was delayed until 3.5 wk of age, when the renal nerves could be identified reliably.

The results of our immunohistochemical studies in the obstructed kidneys are consistent with the renin mRNA accumulation. Chronic UUO in saline-treated rats resulted in a significant increase in both %JGA and %LAA with immunoreactive renin, consistent with our previous findings (14). This was associated with an eightfold increase in renin mRNA levels compared to the intact opposite kidneys and the kidneys of the remaining groups. In contrast, in chronically denervated hydronephrotic kidneys, intrarenal renin distribution and gene expression were suppressed to levels not significantly different from those of sham-operated control rats. The specificity of these results was tested by the mechanical denervation studies: Renin gene expression in the obstructed kidneys was suppressed by ipsilateral but not contralateral renal denervation. Compared to guanethidine treatment (Fig. 5), the less complete suppression of renin gene expression in the obstructed kidneys subjected to mechanical denervation (Fig. 7) may be due to incomplete denervation. This is suggested by the variation in norepinephrine content of mechanically denervated kidneys (Table 4).

These results provide novel evidence that renin gene expression in chronically obstructed kidneys of the maturing rats is facilitated by the chronic or long-term presence of intact sympathetic nervous system and renal nerves. On the other hand, the lack of an effect of guanethidine in sham-operated animals on kidney renin synthesis and distribution, despite a comparable degree of denervation, argues against a primary role of the sympathetic nervous system in the regulation of renin gene expression in normal animals. It should be noted that chemical sympathectomy was associated with a greater decrease in %LAA than %JGA in the obstructed kidneys of UUO + G than in those of UUO + S rats. It is possible that after denervation of the obstructed kidneys, the first cells to switch off renin production are those which were recruited during UUO to express renin, located upstream from the glomerulus along the afferent arteriole.

The mechanism(s) underlying the suppression of renin gene expression by sympathetic denervation in the UUO kidneys is unknown. Several possibilities exist. Recent preliminary studies have demonstrated a transient increase in renin gene expression in response to acute beta-adrenergic receptor stimulation with isoproterenol (25). The increase in renin gene expression was preceded by a decrease in renal renin content, suggesting that isoproterenol increased renin secretion rate and depleted renin stores, thereby stimulating renin gene expression (25). Stimulation of renal mechanoreceptors and increased activity of afferent renal nerves have been shown to occur in response to acute rises in ureteral pressure in adult rats (26). It is not known whether these events occur also in chronic UUO in the young animal, and whether an increase in ipsilateral efferent renal nerve activity also occurs. Should these events occur, norepinephrine released from noradrenergic nerve endings may contribute to increased renin gene expression in the obstructed kidneys, presumably via its interaction with the JG cell B_1 receptor-cAMP system. Supporting this possibility, renal adrenergic axons and terminals have been demonstrated to be intact in the severely hydronephrotic kidney (27). Moreover, stimulation of the hydronephrotic kidney microvessels with beta-adrenergic agonists increases renin release, indicating that beta-adrenergic receptors are functionally intact (27). Another possible mechanism for suppressed renin gene expression by sympathetic denervation in UUO involves hemodynamic factors. The role of renal nerves in the vasoconstriction of the kidney with chronic UUO has not been studied

Figure 1. (*A*) Immunocytochemical detection of beta-tubulin in a kidney from a saline-treated sham-operated rat. Beta tubulin immunoreactivity (*arrows*) is found along the outer wall of the afferent arteriole. (×200). Beta tubulin immunoreactivity was absent in the kidneys of guanethidine-treated rats. (*B*) Immunocytochemical detection of tyrosine hydroxylase in an obstructed kidney of a saline-treated rat. Tyrosine hydroxylase immunoreactivity (*arrows*) is localized to the periphery of a major intrarenal artery. Tyrosine hydroxylase immunoreactivity was absent in the kidneys of guanethidine-treated animals.

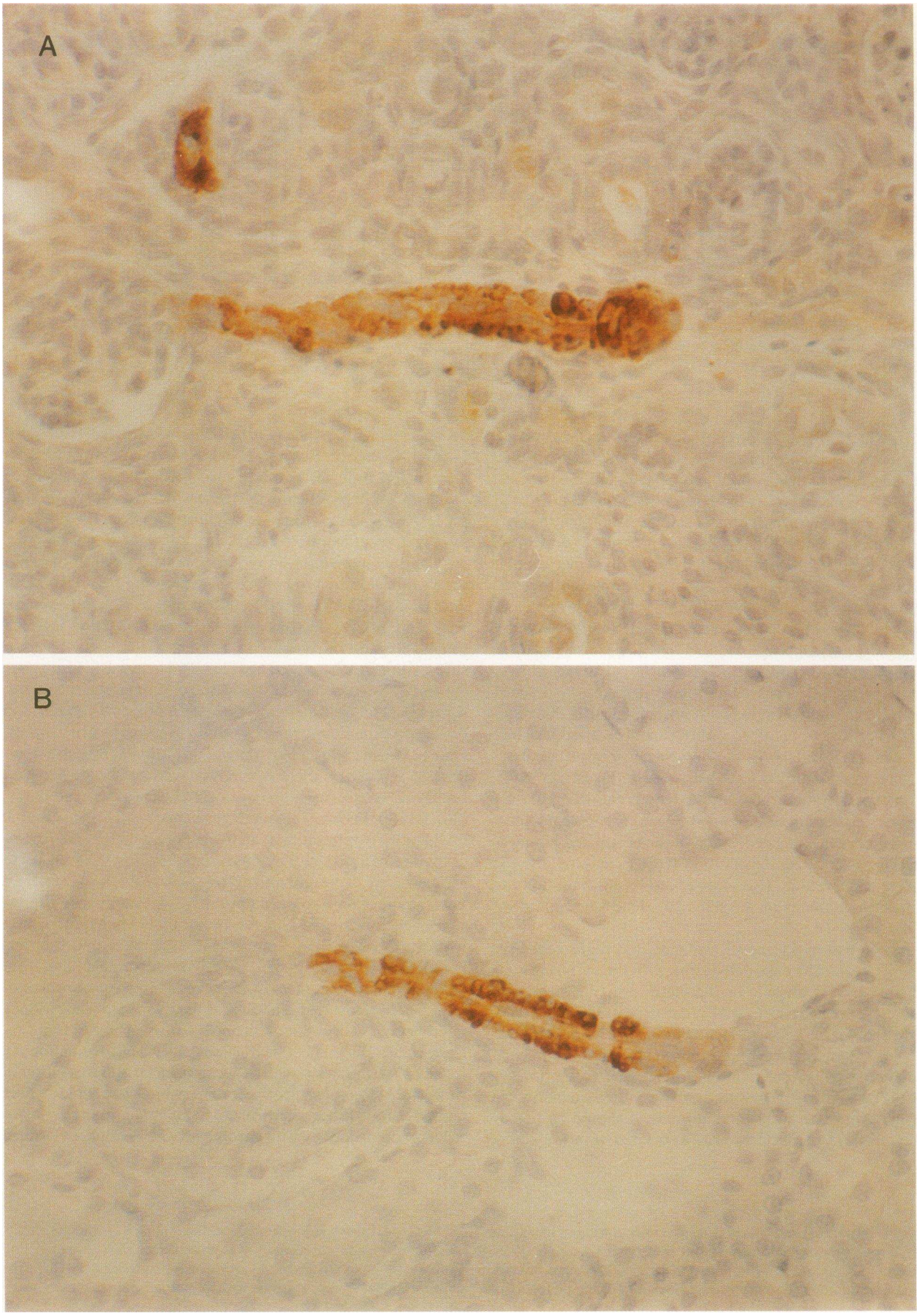
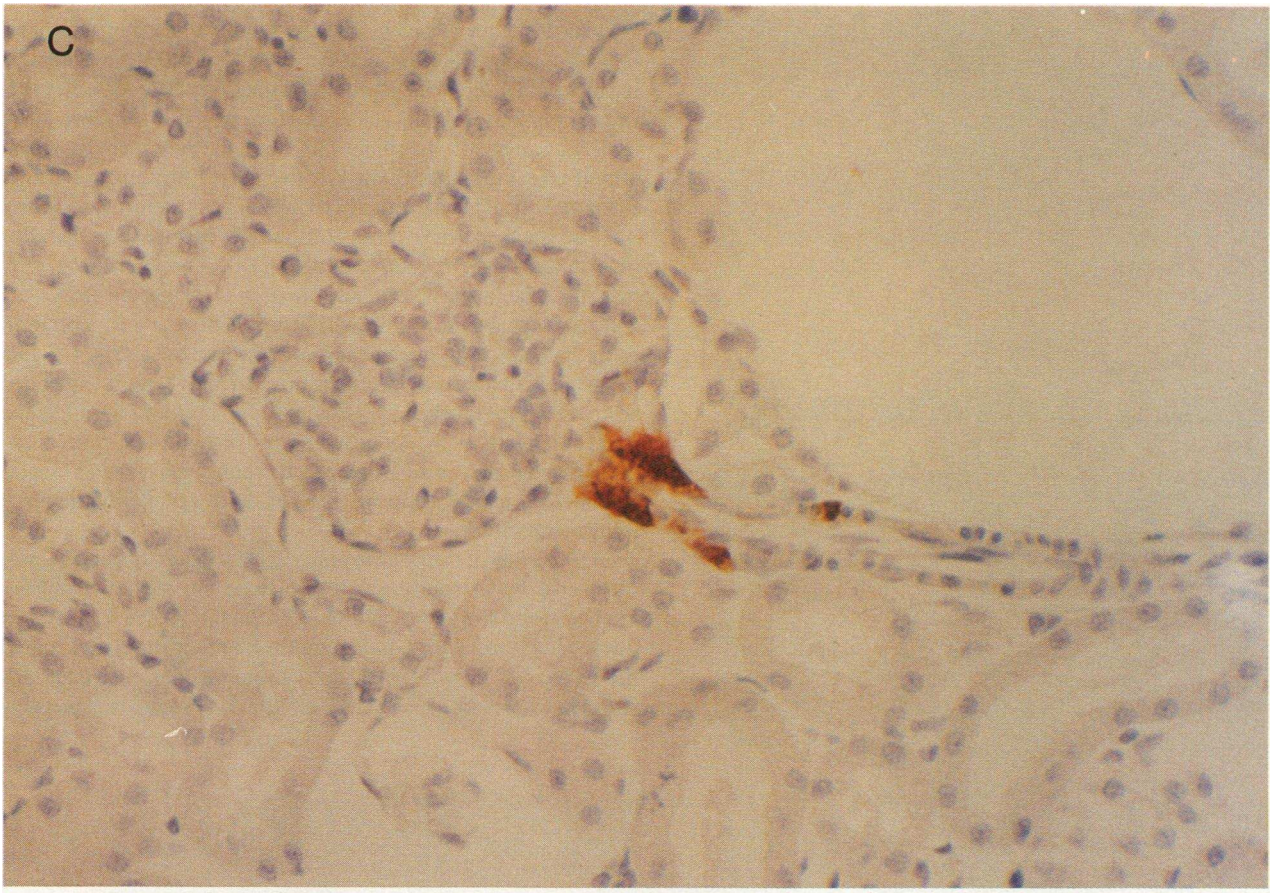
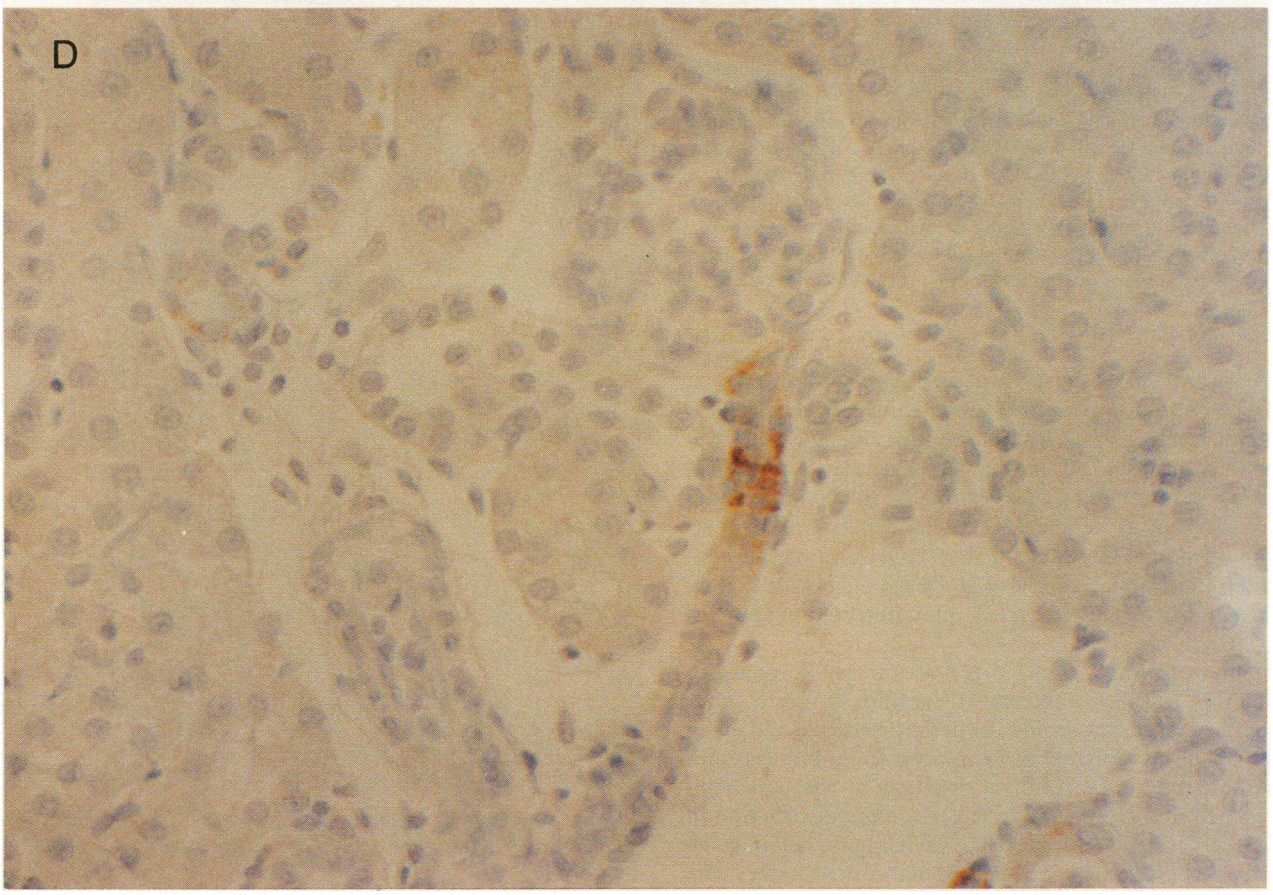


Figure 4. Renin immunocytochemistry. (*A*) Section from an obstructed kidney of a saline-receiving rat (UUO + S group); renin immunoreactivity occupies the entire length of the afferent arteriole. (*B*) Section from the intact opposite kidney of the same animal in *A;* similar to the obstructed kidneys, renin immunoreactivity is found along most of the length of the afferent arteriole. (*C*) Section from an obstructed





kidney of a guanethidine-treated rat (UUO + G); unlike in *A*, renin is no longer seen upstream from the glomerulus and is confined to the vascular pole of the glomerulus. (*D*) Section from the intact opposite kidney of the same animal in *C;* renin immunoreactivity is confined to the vascular pole only (compared to *B*).

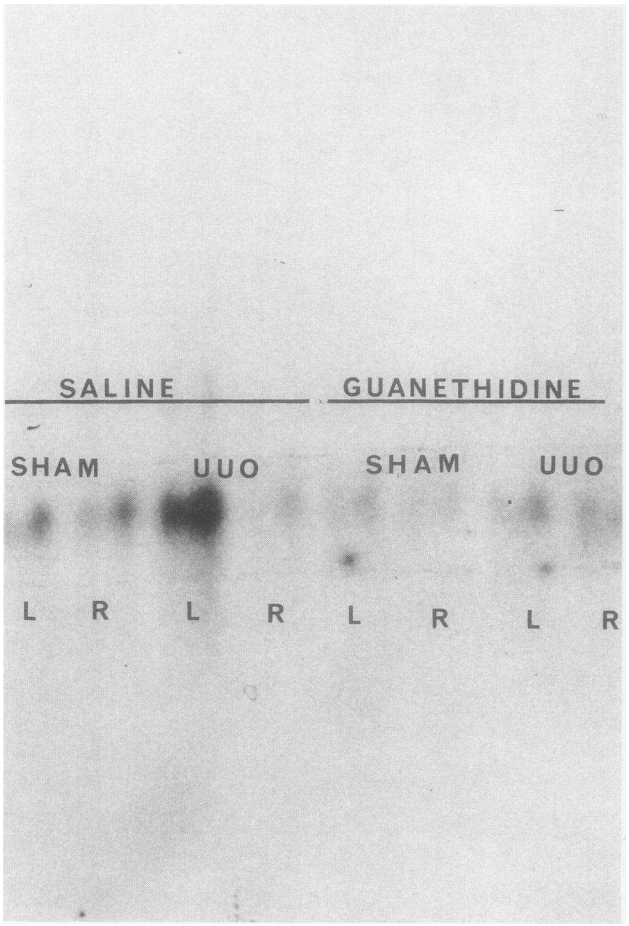


Figure 5. Northern blot. 15 μg of total RNA which was pooled from the left (*L*) and right (*R*) kidneys of six animals in each group have been loaded into each lane and hybridized with a 1.43-kb rat renin cDNA that has been labeled with ^{32}P (see Methods). By densitometric analysis kidney renin mRNA levels were at least eightfold higher in the obstructed kidneys of saline-treated (0.89 absorbance units) than the obstructed kidneys of guanethidine-treated rats (0.08 absorbance units). Denervation by itself did not have a significant effect on renin gene expression in sham-operated kidneys (0.11 absorbance units).

in newborn animals. Although in adult dogs, chemical denervation with 6-hydroxydopamine or alpha-adrenergic blockade do not attenuate renal vasoconstriction (28), a primary effect of guanethidine or ipsilateral mechanical denervation on the vasoconstrictive response to UUO cannot be ruled out. Another possible mechanism is the effect of renal denervation in upregulating intrarenal angiotensin II receptor number and enhancing its intrarenal action (17). These effects ultimately would result in a negative feedback inhibition on renin biosynthesis (29).

We have previously found that 4-wk-old rats subjected to neonatal UUO display an increase in the renovascular distribution of immunostaining renin in both the obstructed and intact opposite kidneys compared to sham-operated kidneys (14). In the present study, bilateral renal denervation with guanethidine resulted in a significant decrease in renin distribution in both the obstructed and intact opposite kidneys. Although efferent renal nerve activity is decreased in the intact kidney with acute contralateral UUO (30), it is not clear whether renorenal reflexes account for the parallel increase in immunoreactive renin in both the obstructed and intact opposite kidneys. It is important to note that a large fraction of the immunoreactive renin contained in the intact kidney in the presence of contralateral UUO may represent inactive renin (prorenin), because the polyclonal renin antibody recognizes both renin and prorenin and because active renin content was depressed in the nonobstructed hypertrophied kidneys (14). These findings may help to explain why vasodilation occurs in these kidneys despite an increase in renin distribution. The lack of increase in renin mRNA in the intact opposite kidney of UUO + S rats despite an increase in renin immunoreactive renin has been addressed previously (14). Possible explanations include the longer half-life of the stored protein (prorenin vs. renin), decreased conversion of prorenin to renin, enhanced translational efficiency of renin mRNA, or renin uptake by the renal microvasculature.

The results of the present study also indicate that compensatory renal hypertrophy was proportionately greater in the intact opposite kidney of UUO + S than UUO + G rats. An interesting finding was that guanethidine treatment attenuated the degree of growth arrest and ureteral dilation in the obstructed kidneys of UUO + G vs. UUO + S rats. It is possible

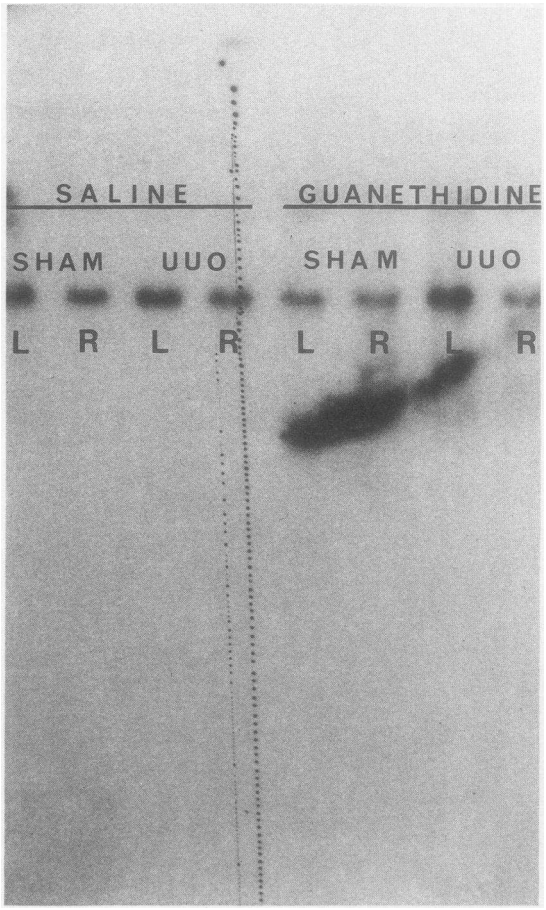


Figure 6. Northern blot hybridized with ^{32}P-labeled beta-actin cDNA after dehybridization of the renin probe. Chemical sympathectomy protocol.

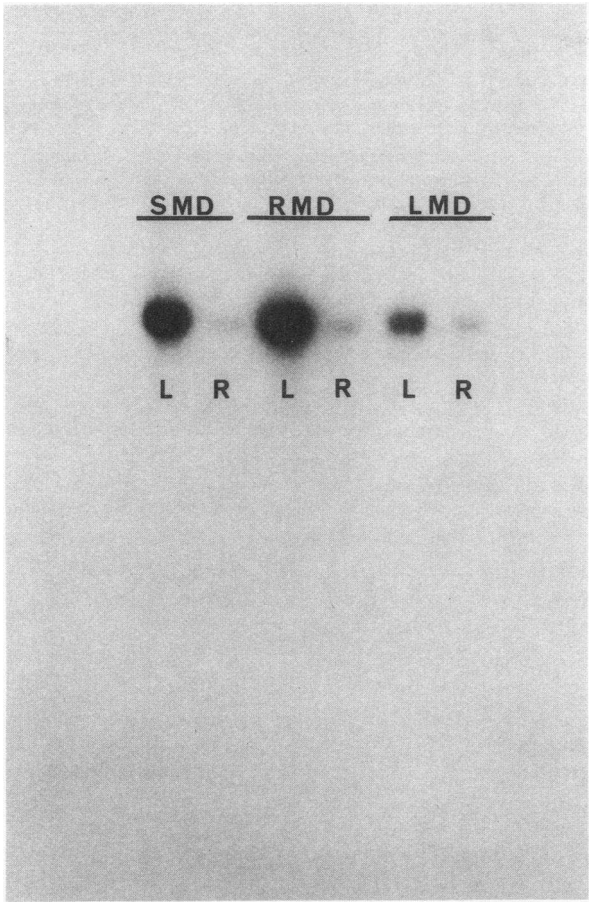


Figure 7. Northern blot hybridized with ^{32}P-labeled renin cDNA. Mechanical denervation protocol. 15 μg of total RNA which was pooled from the left (*L*) and right (*R*) kidneys of each group have been loaded into each lane. LMD, left mechanical denervation (n = 5); RMD, right mechanical denervation (n = 6); SMD, sham-denervation (n = 4). Compared to the obstructed kidneys of SMD rats, renin mRNA levels were fivefold suppressed in the obstructed kidneys with ipsilateral (*LMD*) but not contralateral (*RMD*) mechanical denervation.

that a lower MAP in UUO + G rats may have contributed to the enhanced preservation of renal mass in the obstructed kidneys. Preservation of renal mass did not occur in the mechanically denervated kidneys, but this could be related to the shorter duration of denervation in these kidneys (5–7 d) as compared with the chemically-denervated kidneys (3 wk). Regardless of the mechanism involved, preservation of mass in the obstructed kidneys may have contributed to the reduced compensatory hypertrophy in the intact opposite kidneys of UUO + G relative to those of UUO + S rats. Similar preservation of renal mass in the obstructed kidneys also has been reported in normotensive captopril-treated rats with chronic UUO (11). Taken together, these findings suggest that captopril, by blocking angiotensin II formation, and guanethidine, by decreasing renin synthesis and/or reducing MAP contributed to the preservation of renal mass of the chronically obstructed kidneys. This may have occurred by limiting the effects of angiotensin in producing glomerular hypertension (31) or intrarenal vasoconstriction (8–10).

In summary, chronic UUO in newborn animals was associated with activation of renin gene expression in the obstructed kidneys. Chronic sympathectomy with guanethidine or ipsilateral mechanical renal denervation suppressed renin gene expression in the hydronephrotic kidneys. Sympathectomy with guanethidine also was associated with a decrease in renin distribution in the obstructed kidney as well as the intact kidney with contralateral UUO. In control animals, intrarenal renin distribution and gene expression were not affected by chronic denervation. We conclude that the sympathetic renal nerves modulate renin gene expression of developing kidneys in the setting of chronic ureteral obstruction.

Acknowledgments

Guanethidine monosulfate (Ismelin) has been generously provided by CIBA-GEIGY Pharmaceuticals, Summit, NJ. The authors are grateful to Dr. J. C. Pelayo (University of Colorado School of Medicine) for his help in the mechanical denervation procedure.

Dr. S. El-Dahr is a research fellow of the American Heart Association, Virginia Affiliate. This work was supported by National Institutes of Health grant DK 40558.

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