

Contractility of Renal Cortex following Complete Ureteral Obstruction (38594)

RAY B. NAGLE, LEE W. EVANS, AND DAVID G. REYNOLDS

Divisions of Surgery and Experimental Pathology, Walter Reed Army Institute of Research, Washington, DC 20012

The pathogenesis of the progressive renal interstitial fibrosis common to a variety of chronic renal diseases remains unsettled. In several of these diseases, such as chronic pyelonephritis, the parenchyma is destroyed and the end result is a fibrotic kidney reduced in size.

The interstitial cells of control mammalian kidneys have been shown to morphologically resemble resting fibroblasts (1, 2). In rabbit kidneys injured by complete unilateral ureteral obstruction the interstitial fibroblasts undergo transformation to form cells containing numerous actin-like cytoplasmic myofilaments as well as antigens which cross react with antismooth muscle antibody (3). These changes are similar to those which have been described in contractile granulation tissue (4). This study describes the contractile response to pharmacologic stimulation of renal cortical tissue injured by complete ureteral obstruction. This pathophysiological response may be relevant to the changes which occur in chronic renal injury.

Materials and Methods. Fourteen white New Zealand male rabbits,¹ 2-3 kg, were anesthetized with intravenous pentobarbital, 25 mg kg⁻¹, and the right ureter was exposed through a small suprapubic abdominal incision. This ureter was doubly ligated with 3-0 silk just above the uretero-vesical junction. Half of the animals were studied 8 days following ureteral ligation and the other half 32 days following the operation. Four kidneys from two nonoperated control animals were also studied. The animals were sacrificed by cervical dislocation and both, the right (obstructed) and left (unobstructed) kidneys were removed rapidly and sliced

into 4 mm sagittal sections. The capsule was removed and a peripheral rim of cortex was dissected free above the arcuate arteries and placed in Tyrode's solution of the following composition in g/L: NaCl-8; NaHCO₃-1; KCl-0.2; CaCl₂-0.15; NaH₂ PO₄·H₂O-0.05; MgCl·6H₂O-0.2; dextrose-1. Tyrode's solution of the same composition was used as the bathing fluid in the isolated tissue chamber. The cortical strips were trimmed to 4 mm × 4 mm × 3 mm long and attached by one end to a glass rod with 4-0 silk sutures. Each preparation consisted of four strips of tissue, two from obstructed kidneys and two from the contralateral control kidneys. The cortical strips were then transferred to the tissue chamber which contained Tyrode's solution maintained at 37 ± 1° and bubbled with a gas mixture of 95% O₂ and 5% CO₂. The free ends of the strips were attached to force displacement transducers (FT-.03, Grass Instrument Co., Quincy, MA) which had been previously calibrated with 1 g wt. The signals from these transducers were recorded on a Sanborn recorder. Contractile responses were recorded as millimeters of deflection and converted to milligrams of tension per centimeter of tissue length.

Following a 1 hr equilibration period, tissue responses to norepinephrine bitartrate (NE), angiotensin II (ANG), and 5-hydroxytryptamine creatinine sulfate (5 HT) were determined. The drugs were employed at the dosages shown in Table I and expressed as drug base per ml of bathing solution. Upon completion of the drug sequence the tissues were incubated for one hour in phenoxybenzamine (POB), 10⁻⁸ g/ml, rinsed in fresh Tyrode's solution and the sequence of drug exposures repeated. The differences between responses of the obstructed vs. nonobstructed specimens and the difference between responses before and after alpha adrenergic blockade were assessed for significance with the *t* test for paired data. The unpaired *t* test was employed to test for

¹ In conducting the research described in this report the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences—National Research Council.

TABLE I. CONTRACTILE RESPONSES OF RENAL CORTICAL STRIPS FOLLOWING 8 AND 32 DAYS OF COMPLETE URETERAL OBSTRUCTION

Dose, g/ml		Norepinephrine			5 Hydroxytryptamine		
		5×10^{-7}	5×10^{-6}	10^{-5}			
8	Exp	35.0 ± 9.4 ^a	44.7 ± 8.0	50.3 ± 10.7			
	Cont	1.1 ± 1.1	4.0 ± 1.4	5.9 ± 2.2			
	Day post POB						
	Exp	18.8 ± 6.9	19.9 ± 6.4	24.3 ± 4.7			
32	Cont	1.1 ± 1.1	0.7 ± 0.7	2.9 ± 1.4			
	Exp	16.6 ± 2.2	36.8 ± 4.6	37.2 ± 8.4			
	Day post POB						
	Cont	3.1 ± 1.9	13.1 ± 3.4	14.7 ± 3.2			
32	Exp	5.6 ± 1.2	16.9 ± 1.6	25.2 ± 3.7			
	Cont	0	4.6 ± 2.5	10.0 ± 2.5			
	Day post POB						
	Exp	32.4 ± 4.9	40.7 ± 6.4	51.6 ± 2.9			6.6 ± 3.2
8	Cont	0.6 ± 0.6	3.7 ± 1.7	5.1 ± 1.9	0	1.3 ± 1.3	
	Day post POB						
	Exp	40.4 ± 6.8	52.8 ± 9.4	58.2 ± 13.5	13.7 ± 2.4	21.0 ± 6.1	
	Cont	0	0.7 ± 0.7	2.5 ± 1.2	0	1.1 ± 1.1	
32	Exp	34.0 ± 7.0	49.4 ± 10.2	37.2 ± 16.2	15.0 ± 2.6	12.1 ± 1.9	
	Cont	2.6 ± 1.2	3.4 ± 1.2	1.7 ± 1.7	0	0	
	Day post POB						
	Exp	31.7 ± 2.9	39.7 ± 4.0	51.2 ± 7.3	3.8 ± 1.7	10.7 ± 4.5	
32	Cont	3.1 ± 1.6	4.2 ± 1.1	5.0 ± 2.6	0	0	
	Day post POB						
	Exp	32.4 ± 4.9	40.7 ± 6.4	51.6 ± 2.9	6.6 ± 3.2	15.8 ± 2.7	
	Cont	0.6 ± 0.6	3.7 ± 1.7	5.1 ± 1.9	0	1.3 ± 1.3	

^a Response in mg of tension/cm of length, mean ± SE, *N* = 7.

Post POB = responses following alpha adrenergic blockade with phenoxybenzamine.

statistical significance between the 8- and 32-day experimental groups. At the end of each experiment the strips of tissue were measured and then fixed in 10% neutral buffered formalin and examined by routine histologic methods.

Results. Histological studies of the obstructed kidney strips confirmed the earlier reported findings (5) and consisted of increased cortical interstitial space with increased numbers of fibroblasts at 8 days and diffuse expansion of the interstitial space with greatly increased numbers of fibroblasts, monocytic cells, and fuchsinophilic collagen fibers at 32 days. Control strips revealed no abnormality by light microscopy.

The contractile responses are given in Table I. Eight days following ureteral ligation NE induced contractile responses in the experimental cortical strips that were sig-

nificantly greater than the minimal responses of control strips (*P* < 0.01). Following exposure to phenoxybenzamine, the responses to norepinephrine were attenuated (*P* < 0.05). Cortical strips from kidneys obstructed for 32 days exhibited less contractile activity than did the 8-day specimens. However, strips of the contralateral cortex exhibited greater contractile activity in comparison to the 8-day control specimens; but the responses remained significantly below the responses of the 32-day obstructed specimens (*P* < 0.025). ANG and 5-HT also caused contractile responses of cortical strips from both 8- and 32-day obstructed kidneys. These responses were not changed by alpha adrenergic blockade. Control tissues demonstrated only minimal responses to ANG and negligible responses to 5-HT.

Cortical strips from both kidneys of two

normal rabbits were evaluated for contractile responses in the same manner as the experimental rabbit kidneys. Neither NE, ANG, nor 5-HT caused measurable contractions in these samples.

Discussion. The response to norepinephrine appears to involve an alpha adrenergic receptor mechanism since this response was significantly attenuated by incubating the tissue in Tyrode's solution containing the alpha adrenergic blocking agent phenoxybenzamine. There appeared to be additional receptor mechanisms present which respond to angiotensin II and serotonin which were not affected by phenoxybenzamine.

The results of this study also suggested that there was development of contractility at 32 days in the contralateral, nonobstructed kidney. The data did not provide an explanation for this observation.

The renal cortical strips used in these studies contain intralobular vessels, arterioles, glomeruli, peritubular capillary plexus, cortical tubules, and interstitial cells. Of these components the blood vessels are the only element which would normally be expected to possess significant contractile capabilities. The intralobular arteries are the largest vessels present, but their axis runs perpendicular to the long axis of the strips and it is difficult to envision how contraction of these elements could explain the results obtained. In addition, there were no morphologic changes in the vessels of the obstructed kidney which could explain the increased development of contractility and cortical strips from normal rabbits containing the same vascular components failed to contract. However, the possibility of an increased reactivity of these vessels has not been evaluated.

The interstitial cells showed a dramatic morphologic change in the obstructed kidneys which could explain the development of contractility (3). A similar sequence of events occurs in granulation tissue of healing wounds. The fibroblasts develop actin-like filaments (4), smooth muscle antigens (4), and the ability to contract when stimulated by norepinephrine, bradykinin, serotonin, histamine, and angiotensin (6).

The idea that the renal interstitium can contract when stimulated to undergo transformation by ureteral ligation was predicted on the basis of the ultrastructural morphology of these cells. The fact that there was decreased responsiveness in the kidneys at 32 days is best explained by the progressive increase of interstitial collagen synthesized and deposited by the stimulated fibroblast between 8 and 32 days.

The result of the development in the renal cortex of a network of contractile interstitial cells might become expressed in terms of increased resistance to cortical blood flow. This may explain the profound reduction in blood flow demonstrated by Ibdohrn in rabbits with chronic ureteral obstruction (7). The local ischemia produced by such an effect could also explain the reduction in renal function and the correlated degenerative changes seen within the various portions of the nephron previously described in this obstructive model (5). Diseases such as pyelonephritis, certain toxic drug reactions, and ischemic renal injury may be accompanied by similar interstitial cell responses.

Summary. Contractility of strips of renal cortex obtained from rabbit kidneys obstructed by complete ureteral ligation for 8 and 32 days was tested and compared with control tissue. Concentration-response data were obtained for norepinephrine, angiotensin, and 5-hydroxytryptamine, and were expressed as mg tension/cm of tissue. Control tissue tested simultaneously revealed minimal responses, but obstructed tissue containing modified interstitial cells revealed statistically significant evidence of contraction in response to all three drugs studied. The response to norepinephrine could be blocked by the alpha adrenergic antagonist, phenoxybenzamine, but this drug did not significantly affect the response to angiotensin or 5-hydroxytryptamine. The findings are related to similar observations reported in stimulated fibroblasts of granulation tissue and are discussed in terms of the possible role this phenomenon may play in the pathogenesis of renal interstitial injury.

1. Bulger, R. W., and Trump, B. F., *Amer. J. Anat.* **118**, 685 (1966).

2. Bulger, R. E., and Nagle, R. B., *Amer. J. Anat.* **136**, 183 (1973).
3. Nagle, R. B., Kneiser, M. R., Bulger, R. E., and Benditt, E. P., *Lab. Invest.* **29**, 422 (1973).
4. Gabbiani, G., Hirschel, B. J., Ryan, G. B., Statkov, P. R., and Majno, G., *J. Exp. Med.* **135**, 119 (1972).
5. Nagle, R. B., Bulger, R. E., Cutler, R. E., Jervis, H. R., and Benditt, E. P., *Lab. Invest.* **28**, 456 (1973).
6. Majno, G., Gabbiani, G., Hirschel, B. J., Ryan, G. B., and Statkov, P. R., *Science* **173**, 548 (1971).
7. Ibdohrn, H., and Muren, A., *Acta Physiol. Scand.* **38**, 200 (1956).

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