Contribution of Renal Innervation to Hypertension in Rat Autosomal Dominant Polycystic Kidney Disease

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The kidney has both afferent (sensory) and efferent (sympathetic) nerves that can influence renal function. Renal innervation has been shown to play a role in the pathogenesis of many forms of hypertension. Hypertension and flank pain are common clinical manifestations of autosomal dominant (AD) polycystic kidney disease (PKD). We hypothesize that renal innervation contributes to the hypertension and progression of cystic change in rodent PKD. In the present study, the contribution of renal innervation to hypertension and progression of renal histopathology and dysfunction was assessed in male Han:SPRD-Cy/+ rats with ADPKD. At 4 weeks of age, male offspring from crosses of heterozygotes (Cy/+) were randomized into either 1) bilateral surgical renal denervation, 2) surgical sham denervation control, or 3) nonoperated control groups. A midline laparotomy was performed to allow the renal denervation (i.e., physical stripping of the nerves and painting the artery with phenol/alcohol). Blood pressure (tail cuff method), renal function (BUN) and histology were assessed at 8 weeks of age. Bilateral renal denervation reduced the cystic kidney size, cyst volume density, systolic blood pressure, and improved renal function (BUN) as compared with nonoperated controls. Operated control cystic rats had kidney weights, cyst volume densities, systolic blood pressures, and plasma BUN levels that were intermediate between those in the denervated animals and the nonoperated controls. The denervated group had a reduced systolic blood pressure compared with the operated control animals, indicating that the renal innervations was a major contributor to the hypertension in this model of ADPKD. Renal denervation was efficacious in reducing some pathology, including hypertension, renal enlargement, and cystic pathology. However, sham operation also affected the cystic disease but to a lesser extent. We hypothesize that the amelioration of hypertension in Cy/+ rats was due to the effects of renal denervation on the renin angiotensin system. Exp Biol Med 233:952–957, 2008

Key words: polycystic kidney disease; Cy/+ rat; denervation; hypertension.

Introduction

Inherited renal cystic diseases are common and the fourth leading cause of chronic kidney disease requiring renal replacement therapy. While there are several inherited forms of renal cystic disease, the most common is autosomal dominant (AD) polycystic kidney disease (PKD) with an incidence of approximately 1:500–1:1000. Clinical complications of ADPKD include hypertension, flank pain, arterial aneurysms, and hernias. At present, there are no treatments proven to slow the progression of human ADPKD; however, the hypertension and flank pain require therapeutic intervention.

Hypertension and flank pain are also important contributors to the morbidity of PKD and suggest the involvement of renal innervation. Obviously, pain associated with cystic change would involve renal afferent (sensory) nerves and is more common than previously appreciated (1). Sometimes this pain can be severe, and one treatment has been the surgical unroofing of cysts. This

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procedure has been associated with a transient decrease in blood pressure (2, 3), and an improvement in renal function was described in a few studies (4, 5). Renal innervation, both afferent (sensory) and efferent (sympathetic), is known to contribute to the development and maintenance of numerous forms of hypertension (6–8). The sympathetic nervous system has already been implicated in the hypertension of human ADPKD (9). Renal denervation (10, 11) and thoracoscopic splanchnicectomy (12) performed on ADPKD patients with chronic pain successfully relieved their pain. There are also well-defined renorenal reflexes (13), where renal afferent activity affects efferent activity and *vice versa* (14). Both renal afferent and efferent innervations have been implicated in the hypertension associated with renal failure (15). However, we hypothesized that renal innervations may also contribute to the progression of the renal cystic change as well as the hypertension. To test this we performed a series of bilateral renal denervations in male Han SPRD-Cy/+ rats, a frequently used model of ADPKD (16).

Material and Methods

Han SPRD:-cy/+ Rats. A colony of Han SPRD-Cy_{IU} rats has been maintained in the Laboratory Animal Resource Center of Indiana University School of Medicine (Indianapolis, IN) for more than a decade. The male Cy/+ rats have maintained the significantly severe phenotype similar to that previously described (16). All studies were approved by the Indiana University School of Medicine IACUC committee. Known heterozygous male and female rats were crossed. Their offspring include two different cystic phenotypes. Homozygous cystic rats (Cy/Cy, both male and female) typically succumb to renal failure in their third to fourth postnatal week, so they were euthanized at 21 days of age. Heterozygous (Cy/+) rats develop a slowly progressive form of PKD (16); however, male Cy/+ rats develop renal pathology faster than the females. Therefore, only male rats were utilized in the present study. Litters also contain normal (+/+) rats, some of which were also subjected to bilateral renal denervation procedures. Controls for the denervation procedure were 1) sham-operated rats (subjected to the anesthesia and laparotomy, but without disruption of the renal innervation) and 2) nonoperated rats that were similarly handled but were not anesthetized or subjected to surgery.

Renal Denervation. *Bilateral Renal Denervation.* Male rats from entire litters of pups from heterozygote crosses were randomly subjected to either 1) a bilateral renal denervation or 2) a sham operation (using a procedure previously described) (17). A third group was added midway through the study that included nonoperated control rats because the severity of the disease in the operated control rats appeared to be diminished compared with historical data on these rats. These groups all included both cystic (Cy/+) and normal (+/+) offspring. Isoflurane anesthetized 4-week-old males were subjected to a mid-sagittal abdominal incision. Renal denervations were performed as previously described (17). Briefly, the renal arteries were separated from associated structures, and renal nerves that run along the artery identified and severed. A piece of parafilm was slipped around the renal artery and a thread saturated with 10% phenol in 95% ethanol was inserted around the renal artery. The thread was kept moist with the solution for 5 minutes and removed prior to closing the abdomen. The abdomen was closed in 2 layers (fibromuscular and skin) using interrupted silk suture for the fibromuscular layer and stainless steel clips for the skin. Animals were evaluated at 8 weeks of age. The evaluation included weighing the rat and measuring systolic blood pressure using a tail cuff system (IITC Inc., tail cuff plethysmography system, Woodland Hills, CA). Blood pressure was measured at an ambient temperature of 27°C in an enclosed cabinet with low intensity white noise (a small fan) and mild restraint. The rats were anesthetized again (sodium pentobarbital, 100 mg/kg, ip) and blood collected (for the determination of serum urea nitrogen concentration-SUN). The left kidney was removed and weighed while the right kidney was perfusion fixed with 4% paraformaldehyde in 0.1 M phosphate buffer. The right kidney was then weighed and sections processed for paraffin embedment, sectioning, and staining (periodic acid Schiff reaction method) as described previously (18). The volume density (Vv) of the cystic space was determined using point count stereology (19). Cyst Vv, the degree of cystic change, in renal cortex and outer medulla was determined. Any open space larger than about 20 μm was considered a cyst. Since some blood vessels had open spaces greater than 20 μm, the value obtained from the normal rats was averaged and subtracted from the values obtained from the cystic rats to compensate for the vascular space.

The evaluation of the denervation procedure was accomplished using the sucrose-phosphate-glyoxylic acid method for microscopic histofluorescent evaluation of monoaminergic nerves as previously described (20).

Data Evaluation. Data are expressed as mean ± standard error of the mean. The data derived from the bilateral renal denervation studies was subjected to an analysis of variance with a $P < 0.05$ indicating significance.

Results

Bilateral Renal Denervation. The bilateral renal denervation procedure did not result in a body weight difference in normal or cystic rats (normal rats, nonoperated controls, 303.6 ± 2.9 grams; bilateral denervated, 288.9 ± 10.9 grams; 294.1 ± 5.2; and 286.3 ± 4.6 for nonoperated and bilateral denervated cystic rats). In all three groups, the cystic kidneys were larger than their normal counterparts, expressed as either total weight or as kidney weight as a percent of total body weight (Table 1). Performing a laparotomy reduced the size of the cystic kidney as evinced

Table 1. Effects of Bilateral Renal Denervation in the Cy/+ Rat

Group	Phenotype	Total kidney weight (g)	Kidney wgt as % body wgt	Cyst Vv (%)	BUN (mg/dl)	Systolic BP (mmHg)
Renal DeNx	Normal (4)	3.08 ± 0.15	1.07 ± 0.05		19.6 ± 2.6	134 ± 6.3
	Cystic (8)	5.59 ± 0.60[a]	2.00 ± 0.20[a]	17.2 ± 5.2[a]	23.0 ± 2.6[a]	123 ± 8.5[a,b]
Operated ctrls	Normal (4)	2.57 ± 0.30	1.00 ± 0.07		20.5 ± 1.2	127 ± 1.3
	Cystic (8)	6.96 ± 0.59[a]	2.34 ± 0.20[a]	29.6 ± 3.9	33.1 ± 3.7[a]	155 ± 6.0
Nonoperated ctrls	Normal (4)	3.00 ± 0.10	0.99 ± 0.04		25.9 ± 1.1	133 ± 5.3
	Cystic (9)	9.12 ± 0.36	3.11 ± 0.14	45.1± 7.0	47.9 ± 1.1	161 ± 6.0

[a] $P < 0.05$ for difference from nonoperated controls.
[b] $P < 0.05$ for difference from operated controls; mean ± SEM (n).

by a reduced cystic kidney size in both the renal denervated and operated control cystic rats (Table 1). However, volume density of renal cysts was only significantly reduced in the renal denervated rats, with Vv of cysts in operated control rats having an intermediate value not different from either denervated rats or nonoperated sham controls (Table 1 and Fig. 1). Blood urea nitrogen concentrations were significantly lower in both the operated control and renal denervated cystic rats compared with the nonoperated control cystic rats; however, the absolute value for the operated control rats was again intermediate between the values for the denervated and nonoperated animals. Control cystic rats (operated or nonoperated) were hypertensive (Table 1). Denervated cystic rats had a lower systolic blood pressure as compared with both operated and nonoperated control cystic rats (Table 1). In operated control cystic rats, the surgical procedure, a laparotomy, reduced the severity of the cystic renal enlargement (reduced kidney weight compared with nonoperated control rats). However, a reduction of systolic blood pressure was the only change that was specifically associated with the actual bilateral renal denervation versus operated control cystic rats. Renal denervation reduced BUN and blood pressure to normal levels in cystic rats, suggesting a role for renal innervation in both hypertension and renal dysfunction associated with ADPKD.

The ability to lose monoaminergic innervation using this surgical procedure was confirmed in a group of rats sacrificed a week after the bilateral renal denervation. Innervation by sympathetic nerves was evaluated using the sucrose-phosphate-glyoxylic acid (SPG) histofluorescence method (20). By 8 weeks of age, the renal innervation was redeveloping in the cortex of the denervated rats.

Discussion

The most common complications associated with ADPKD that require medical intervention are renal pain, hypertension, and uremia. The pain is typically associated with more advanced cystic change; however, it may be more common and occur earlier than previously appreciated (1). Pain associated with PKD may modulate other facets of the disease through a renorenal feedback loop involving the sympathetic nervous system. Hypertension can develop in earlier stages of the disease and contributes to the morbidity and mortality of ADPKD.

Renal innervation has long been shown to play a role in the pathogenesis of numerous forms of hypertension in the rat (6–8). Renal denervation normalized the blood pressure in male Cy/+ rats compared with operated control rats (who exhibited a similar reduction in renal size). Therefore, renal innervation appears to be a major factor responsible for the elevated blood pressure in this PKD model. Studies have shown that renal denervation reduces renin expression (21), therefore the renal denervation of the Cy/+ rats most likely reduced renin expression. Renal denervation also reduces the effects of angiotensin II (22). Hypertension in human ADPKD has been closely associated with an elevation of the renin-angiotensin system (23–25) and is often treated with angiotensin converting enzyme (ACE) inhibitors and/or angiotensin receptor antagonist (ARBs). Currently, the NIH-funded HALT Study is evaluating the efficacy of ACE inhibitors and ARBs in slowing the progression human ADPKD (http://www.nih.gov/news/pr/jan2006/niddk-24.htm). Treating male Cy/+ rats with ACE inhibitors ameliorates both the hypertension and the renal cystic disease (26, 27). There is a close relationship between renal innervation and renin expression in both the abnormal kidney (in unilateral ureteral obstruction) (28) and normal kidney development (29). Therefore, the likely mechanism of the denervation-induced reduction in systolic blood pressure in the Cy/+ rat was through its modulation of renal renin release and AII activity. The effects of the denervation are probably due to the renal sympathetic innervation since chronic intrarenal norepinephrine infusion alone can also induce hypertension (30, 31). While the exact mechanism of the denervation affect is not known, the present study implicates renal innervation in the hypertension of ADPKD, at least in the Cy/+ rat.

The decline in renal function and disease progression in human ADPKD appears to be closely linked with the decline in renal blood flow (32). Renal sympathetic innervation as well as increased AII can cause renal vasoconstriction (14), and data suggest that the cystic kidney in the Cy/+ rat already has a compromised renal blood flow (unpublished observations using *in vivo* two-photon microscopy). Therefore, the denervated rats may

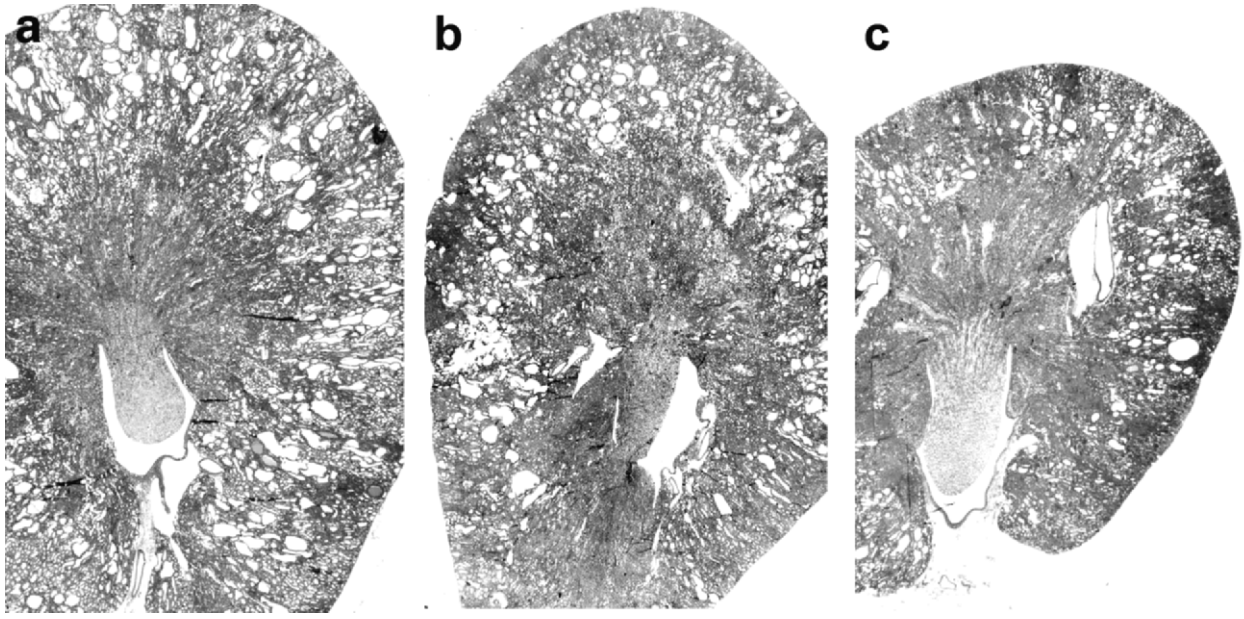

Figure 1. Light micrograph of the kidneys from 8-week-old male Cy/+ rats that were from (a) nonoperated control rat, (b) operated control rat, or (c) a rat in which the renal nerves were removed from both kidneys at 4 weeks of age. The kidneys were notably smaller and less cystic for both the operated control rats and rats subjected to a renal denervation compared with the nonoperated control rat.

have experienced better renal blood flow, limiting or eliminating the deterioration of the renal function associated with their disease. In any event, the renal denervation reduced both the progression of the histopathology and the maintenance of a normal renal function.

Cyst enlargement requires an increased proliferation of renal epithelial cells, a well-recognized feature in cystic kidneys. Angiotensin II is known to be a renal epithelial cell mitogen (33) and may directly contribute to the PKD (26, 27). Angiotensin II can also potentiate the mitogenic effect of epidermal growth factor (34), and the EGF receptor tyrosine kinase has been implicated in Cy/+ induced PKD (35). Alternatively, renal proximal tubules express β adrenergic receptors (36, 37) and a reduction in the activity of this G-protein receptors may be responsible for this amelioration of renal enlargement after denervation The β adrenoceptor is a G-protein receptor that causes activation of adenylate cyclase and the generation of cyclic AMP, a known cystogenic molecule (38, 39). An increase in sympathetic activity in PKD could overstimulate these receptors and cause an increase in cystic epithelial proliferation. The beta adrenoceptor agonist, isoproterenol, can stimulate renal epithelial cell proliferation *in vitro* (40). A third possible explanation for the amelioration in cystic disease in renal denervated rats may result from the lower blood pressure. A published CRISP study found that hypertensive autosomal dominant PKD patients had more cystic change than did normotensive autosomal dominant PKD patients (41). While several explanations are available for the renal denervation reduction in renal cystic change, they do not adequately explain the reduction in cystic renal enlargement seen in operated control rats that still had intact renal innervation.

It is unclear how a laparotomy would lead to a reduction in renal cystic enlargement in the Cy/+ rat. A possible explanation for the reduction in renal cystic enlargement in operated control male Cy/+ rats is the elevation in glucocorticoids associated with the stress of surgery. Glucocorticoid administration at pharmacologic doses caused both a reduction in renal cystic enlargement and cyst volume density in male Cy/+ rat (18). However, in the present study, the operated control rats did not have a statistically decreased cystic pathology. The elevation of cortisol from the surgical stress of sham surgery would not be expected to decrease blood pressure and would probably be temporary. There is also the possibility that renal afferent innervation may be playing a role in the reduction in renal cystic enlargement seen in the operated cystic control rats. The midline abdominal laparotomy incision cut through dermatomes T8 through L1, which include some of the same dermatomes that innervate kidney (42), leading to a perceived increase in renal afferent activity. Because the stimulation of the renal afferent limb of the renorenal reflex causes a reduction in renal sympathetic activity (13), surgery-induced increase in afferent activity could feed back to inhibit renal sympathetic activity. However, this reduction in renal sympathetic activity may be insufficient to affect blood pressure. A CRISP study (41) found that both renal size and hypertension were correlated with the development of renal dysfunction in human autosomal dominant PKD. The laparotomy in sham denervation leads to a reduction in renal size but not the hypertension, suggesting that improving just one factor could partially

inhibit a loss in renal function as was seen in nonoperated control cystic rats.

Findings from the present study suggest that hypertension, which may affect PKD progression, is under the influence of the sympathetic nervous system. Renal sympathetic innervation probably acts through the renal regulation of renin synthesis to cause the hypertension, at least in part. While the exact mechanism is unknown, renal denervation also inhibited the progression of the renal cystic disease and the deterioration in renal function. These data support an important contribution of renal innervation to the progression of this chronic kidney disease.

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