Effects of Acute Renal Denervation on Kidney Function in Deoxycorticosterone Acetate–Hypertensive Swine

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SUMMARY
Deoxycorticosterone acetate-induced hypertension in Yucatan miniature swine appears to involve elevated peripheral sympathetic activity. Abnormalities in renal function in these hypertensive animals are also apparent. To determine the extent to which renal nerve activity controls kidney function in animals with established deoxycorticosterone acetate hypertension, the effects of acute renal surgical denervation were assessed in five normal and 10 deoxycorticosterone acetate–treated swine. After 12 to 16 weeks of treatment, mean arterial pressure rose from the normal level of 110 to 120 to 164 ± 4 mm Hg but was decreased to 131 ± 4 mm Hg by anesthesia. In the normal animals, blood pressure under anesthesia was 114 ± 9 mm Hg. Acute left kidney surgical denervation significantly decreased renal vascular resistance and increased renal blood flow, glomerular filtration rate, urine flow, and sodium excretion only in the treated animals. In an additional group of six normal and eight deoxycorticosterone acetate–treated swine, the responses to renal pharmacological denervation with intrarenal guanethidine were evaluated. Guanethidine had no significant effect on renal blood flow, vascular resistance, glomerular filtration rate, urine flow, or sodium excretion in the normal animals. In contrast, in the mineralocorticoid–hypertensive animals, guanethidine significantly decreased renal vascular resistance and caused a diuresis and natriuresis with no change in glomerular filtration rate. We conclude that, in deoxycorticosterone acetate–treated miniature swine with established hypertension, renal nerve activity appears to be elevated and important in determining renal hemodynamics and sodium and water excretion. (Hypertension 8: 925–931, 1986)

KEY WORDS • hypertension • renal nerves • Yucatan miniature swine • renal hemodynamics • guanethidine • sodium excretion

It has become clear that the kidneys play a pivotal role in the development and maintenance of hypertension. Studies have also indicated that the renal nerves appear to be directly involved. Chronic renal denervation attenuates the development of hypertension in various hypertensive animal models. If renal nerve activity is elevated, it could affect this process either by controlling renal hemodynamics or the release of renin or other vasoactive compounds or by directly influencing renal tubular sodium reabsorption. In some studies in which renal denervation lowered blood pressure, there was an associated increase in sodium excretion, whereas in others the hypotensive effect of denervation did not alter sodium balance. This latter finding and studies using renal hypertensive and other animal models suggest an importance of the renal afferent nerves. Renal denervation may interrupt renal afferent input to the central nervous system, resulting in a decrease in sympathetic tone.

Studies from our laboratory have been concerned with evaluating cardiovascular and renal function in a relatively novel hypertensive model, the two-kidney deoxycorticosterone acetate (DOCA)–treated Yucatan miniature swine (YMS). We have previously shown that these animals have a high level of peripheral sympathetic activity that is involved in maintaining the hypertensive state through a selective influence on total peripheral resistance. This feature may be in contrast to the DOCA-salt and spontaneously hypertensive rat models of hypertension, in which the sympathetic system is believed to play a small role once the hypertension has been established. The DOCA-hypertensive YMS have increased renal vascular resistance, and despite a 40 to 60 mm Hg increase in mean renal perfusion pressure, glomerular filtration rate (GFR)
and sodium excretion are similar to those seen in control animals. These functional abnormalities may be mediated by augmented renal nerve activity.

The purpose of this study was to determine the effects of acute surgical and pharmacological renal denervation on kidney function in DOCA-hypertensive and normal YMS. The rationale for this study was that if renal sympathetic nerve activity is elevated and influencing renal function in the established phase of hypertension in DOCA swine, then renal denervation should produce greater renal vascular and excretory changes in the DOCA-treated than in the normal animals.

Materials and Methods

Animals and Deoxycorticosterone Acetate Implantation

Male and female YMS were obtained from Buckshire Corporation, Perkasie, PA, USA. Animals were approximately 8 to 12 months of age at the time the study started and ranged in weight from 35 to 75 kg. All animals were fed a standard swine ration diet that contained approximately 200 mEq of sodium per day. Water was provided ad libitum. Of the 29 animals used for this study, 18 pigs, selected at random, were implanted with DOCA-impregnated silicone strips (100 mg/kg) according to the procedure of Terris et al. Under sterile conditions and with the YMS under halothane anesthesia, strips were implanted subcutaneously in the animal’s right flank. The strips remained in the DOCA-treated pigs for 3 to 4 months before the study began. Eleven nonimplanted pigs served as controls. We previously evaluated the effects of silicone implants that did not contain DOCA. When we compared control animals (untreated) with animals receiving silicone implants (no DOCA), we found no differences in blood pressure, renal hemodynamics, electrolyte excretion, or any hematological parameters. The silicone implant appears to be relatively inert. The strips are implanted subcutaneously through a 2- to 3-cm incision. In chronically implanted animals (DOCA strips for 6-12 months), we have seen no signs of infection or irritation.

Approximately 1 week before the study began, all DOCA-treated YMS were anesthetized with halothane and a catheter was placed in a carotid artery and exteriorized on the animal’s back. To confirm and quantify the hypertensive state, mean arterial pressure (MAP) was determined in the conscious animals before the renal function studies began. Arterial pressure was measured by connecting the arterial catheter to a Statham pressure transducer (Oxam, CA, USA). Pulsatile and electronically averaged arterial pressure were recorded on a Grass direct writing recorder (Quincy, MA, USA).

Surgical Preparation for Renal Function Studies

At the time of the renal function studies, DOCA-treated and normal animals were anesthetized with sodium pentobarbital (25 mg/kg). Ketamine hydrochloride (4 mg/kg, i.m.) was used as a preanesthetic. The trachea was intubated, and the animal was mechanically ventilated. Polyethylene catheters were placed in the jugular vein and in the abdominal catheters through the femoral artery. The jugular catheter was used to administer a prime (62 mg/kg) and sustaining dose of insulin in 0.9% NaCl at 1 ml/min to achieve a plasma insulin level of 0.2 mg/ml. The femoral catheter was used to obtain blood samples and to monitor MAP and heart rate (HR). The bladder was approached through a ventral midline incision, and both ureters were cannulated. The left kidney was approached through a retroperitoneal flank incision, and an electromagnetic flow probe (BL-5000 series; Biotronex, Kensington, MD, USA) was placed around the left renal artery for the measurement of left renal blood flow (LRBF). The procedures used for DOCA implantation, surgical placement of long-term catheters, measurement of blood pressure in conscious animals, and the surgical and testing protocol for assessing kidney function have been reviewed and approved by the university veterinarian.

Experimental Protocols

After being surgically instrumented as described, 10 DOCA-treated and five normal animals were allowed 45 to 60 minutes to equilibrate. At the end of the equilibration period, a 30- to 40-minute control period was begun; urine was collected from both kidneys and blood samples were obtained at the midpoint of the period. At the end of the control collection, the exposed left kidney was surgically denervated by cutting all visible nerves in the area of the renal hilus and by stripping approximately 1 cm of the adventitia from the renal artery. The area was then painted with a phenol solution (10% phenol in absolute ethanol). The right kidney was untouched. Following left renal denervation, the animals were allowed 20 to 30 minutes to reequilibrate and another collection period lasting 30 to 40 minutes was initiated. A separate group of six normal and eight DOCA-treated swine was prepared as previously described. In addition, small, curved 25-gauge needles were inserted into the left renal artery retrograde to the direction of blood flow. The needle was attached with thin polyethylene tubing to an infusion pump (Model 99H; Razel, Stamford, CT, USA). During a 30- to 40-minute control urine collection period, 0.9% NaCl was infused intrARENALLY at 0.1 ml/min. After completion of this collection, the infusion of saline into the left kidney was changed to guanethidine (GE), 0.5 mg/min, dissolved in the saline. The GE was administered intrarenally for the remainder of the experiment. This dose of GE infused into the left kidney abolished the renal vasoconstriction to carotid occlusion and was found to be noncircular (i.e., no or minimal changes in systemic hemodynamics). This dose and acute administration of GE have been shown to block neurogenically mediated renal tubular sodium reabsorption. Although the mechanism of GE’s action is not clearly understood, GE administration produces effective adrenergic blockade within 20 minutes that is not dependent on changes in tissue catecholamine content.
Monitoring and Analytical Procedures

The MAP and HR, derived from pulsatile arterial pressure, were recorded continuously. The LRBF was monitored and electronically averaged by a Biotronex electromagnetic flowmeter system. Mean LRBF and pulsatile LRBF were recorded on a Grass recorder. Zero flow was determined by mechanically occluding the renal artery distal to the flow probe at the beginning and end of each experiment. At the completion of the experiment, the flow probe and flowmeter system were calibrated in situ by making several timed blood flow collections through a catheter placed in the renal artery distal to the probe.

Both kidneys were removed and weighed at the end of each experiment. The LRBF was corrected for differences in animal and kidney weight by dividing total LRBF (ml/min) by left kidney weight (g), and flow was expressed in milliliters per gram per minute. Left renal vascular resistance (LRVR) (mm Hg/ml/g/min) was calculated as the quotient of MAP (mm Hg) and LRBF (ml/g/min).

The GFR was determined by inulin clearance and was also normalized for kidney weight and expressed as milliliter per 100 gram per minute. Plasma and urine inulin concentrations were determined by the anthrone method. Serum and urine samples were analyzed for sodium and potassium by flame photometry.

Statistical Analysis

All data were analyzed using a repeated-measures analysis of variance for a nested-factorial design. Duncan's multiple range test was used to compare treatment means. Differences were considered significant at a p level of less than 0.05. All values in the text, tables, and figures are listed as the mean ± SE.

Results

In the 10 DOCA-treated animals used for these studies, conscious MAP was 164 ± 4 mm Hg. Pentobarbital anesthesia decreased the MAP significantly to 131 ± 4 mm Hg; MAP in the anesthetized DOCA-treated group was still significantly higher than that in anesthetized controls (114 ± 9 mm Hg).

The effects of left kidney renal denervation on MAP, HR, LRBF, and LRVR are illustrated in Figure 1. A slight increase in MAP, which occurred in the DOCA-treated animals after denervation, was not significant. Although HR was 6% lower in the DOCA-treated animals during the control period (p<0.05), this difference between the groups was abolished after denervation. The LRBF was similar between the two groups before denervation. Renal denervation, which caused no change in LRBF in the normal animals, significantly increased LRBF by 30% in the DOCA-treated YMS. The LRVR was 26% greater in the DOCA-treated than in the normal animals during the control period (p<0.05). Denervation did not alter LRVR in the normal animals but significantly decreased LRVR in the DOCA-treated pigs to a value identical to that seen in control animals.

The changes in the left kidney GFR, urine volume, and electrolyte excretion for normal and DOCA-treated animals before and after denervation are shown in Figure 2A. Before denervation, GFR was significantly lower in the DOCA-treated animals than in the normal YMS. Denervation significantly increased left kidney GFR, urine flow rate, and sodium excretion in the DOCA-treated animals but had no effect on these parameters in normal YMS. Potassium excretion was significantly increased by denervation in the normal YMS but not in the DOCA-treated animals. In both groups, the increases seen in left kidney fractional sodium and water excretion with denervation were not significant.

In the right untouched kidney, left renal denervation tended to decrease GFR, urine flow rate, and sodium excretion for both the normal and DOCA-treated animals (Figure 2B); however, these changes were not significant. Left kidney denervation did not change right kidney potassium excretion in the normal animals, whereas, right kidney potassium excretion was significantly decreased by contralateral denervation in the DOCA-treated animals (see Figure 2B).

The effects of intrarenal GE administration in six normal YMS are shown in Table 1 and Figure 3A. Neither HR nor MAP were altered by GE. Although
LRBF increased and LRVR decreased during GE administration, these changes were not significant in the normal pigs. During the control period, GFR, urine volume, and sodium excretion in the instrumented left kidney of the normal group were similar to that in the untouched right kidney. Potassium excretion, however, was 24% lower in the left kidney ($p < 0.05$). Administration of GE caused insignificant increases in left kidney urine volume and sodium excretion (see Figure 3A). Neither left kidney GFR nor potassium excretion was affected by GE infusion. Administration of GE to the left kidney did not produce any significant changes in right kidney function in the normal animals (see Figure 3A).

The effects of left intrarenal GE administration in the DOCA-treated pigs are shown in Table 1 and Figure 3B. Left intrarenal GE administration did not alter MAP or HR in the DOCA-treated animals. The LRBF,
Figure 3. Effects of left kidney guanethidine treatment on left and right kidney glomerular filtration rate (GFR), urine volume, and sodium excretion in six normal swine (A) and eight DOCA-treated swine (B). Asterisk indicates significant difference (p<0.05) compared with control period.

Discussion

In studies using various hypertensive animal models, the renal nerves have been implicated as being important determinants of renal and cardiovascular function in the early developmental stages of hypertension. The extent to which the renal nerves are important in the sustained phase of hypertension is open to question, with some reports suggesting that in the rat model they play a diminished or negligible role once the hypertension has been established. The purpose of this study was to focus on the latter aspect by determining if renal nerve activity significantly influenced kidney function after 12 to 16 weeks of hypertension in DOCA-hypertensive swine.

Surgical renal denervation increased RBF and decreased LRVR in the DOCA-hypertensive animals but not in the controls. Denervation decreased the LRVR of the DOCA-treated animals to a value identical to that of the controls. Acute surgical renal denervation also caused urine flow rate, sodium excretion, and fractional salt and water excretion to increase in the left kidneys of both normal and DOCA-treated YMS. However, the magnitude of the increments in water and sodium excretion, as well as the increases in left kidney GFR and RBF, was significant only in the DOCA-treated group after denervation. Since these renal functional parameters are known to be directly influenced by the renal nerves, it would seem that renal function in the DOCA-treated YMS is influenced to a greater extent by the renal nerves than in the normal animals, suggesting elevated renal sympathetic nerve activity.

Recent studies from this laboratory involving direct measurement of renal nerve activity have shown that baroreceptor reflex-mediated increases in renal nerve activity are greater in DOCA-hypertensive animals than in normal animals. We have not yet quantitated renal nerve activity in conscious normal versus DOCA-treated animals in order to ascertain if the absolute nerve activity is elevated. There are, however,
several lines of indirect evidence supporting the hypothesis of increased peripheral sympathetic and renal nerve activity in DOCA-treated swine with established hypertension. In this model with established hypertension, the increased pressure is due entirely to increased total peripheral resistance. Circulating catecholamines are elevated in conscious DOCA-hypertensive animals. Hexamethonium bromide and phenolbenzamine decrease MAP by lowering total peripheral resistance.

It could be argued that the experimental conditions (i.e., anesthesia, surgical trauma) were responsible for artifically elevating renal nerve activity. We feel that this is an unlikely explanation since both the normal and DOCA-treated animals were exposed to the same conditions. In addition, despite the common belief that pentobarbital increases peripheral sympathetic activity, several reports including a recent paper by Zimpfer et al. have shown that pentobarbital anesthesia decreased circulating catecholamines and depressed the peripheral sympathetic vascular response to hemorrhage in dogs. In the DOCA-treated swine, anesthesia decreased MAP from 164 ± 4 to 131 ± 4 mm Hg. We believe that this decrease is due to a direct effect of pentobarbital relaxing vascular smooth muscle and lowering peripheral resistance (unpublished observations). This response could evoke an acute baroreceptor-mediated increase in renal nerve activity. It has been shown that, with changes in baseline blood pressure, baroreceptors adapt to the new pressure within minutes. Consequently, over the 3- to 4-hour time course of these experiments, a significant amount of baroreceptor resetting probably occurred. We have recently reported that in DOCA-treated animals the decrease in MAP with anesthesia is extremely variable: some animals show no change, whereas others demonstrate significant declines.

In the group of surgically denervated DOCA-treated animals, the change of blood pressure from conscious to anesthetized conditions ranged from 6 to 39% with a mean change of 20%. If the diuresis or natriuresis observed with renal denervation was dependent on baroreceptor-mediated increases in renal nerve activity caused by a lowering of MAP with anesthesia, those animals showing the greatest declines in MAP with anesthesia should have exhibited the largest decreases in LRVR and increases in urine flow rate and sodium excretion to renal denervation. This was not the case. The correlation coefficients between the percentage changes in MAP with anesthesia and the percentage changes in LRVR, urine flow rate, and sodium excretion with surgical denervation were 0.45 (p = 0.22), 0.34 (p = 0.33), and 0.13 (p = 0.67), respectively. Consequently, the effects of renal denervation in the DOCA-hypertensive animals do not appear to be due to a pentobarbital-induced decrease in MAP and a baroreceptor-mediated increase in renal nerve activity.

In addition to elevated nerve activity in DOCA-hypertensive YMS other factors must be considered in the interpretation of our results. These animals are known to have increased vascular responses to pressor agents, such as norepinephrine or angiotensin II. Also, an increase in renal α-adrenergic receptor density, reported in other hypertensive models, could be responsible for the changes we observed with renal denervation.

In the studies that have shown a decrease in blood pressure following renal denervation with no apparent change in sodium excretion or balance, it has been postulated that the denervation effect may be due to an interruption in renal efferent nerve traffic, which may be important in determining peripheral sympathetic tone and contralateral renal nerve activity. This study did not address this issue directly since surgical denervation eliminated both efferent and afferent nerve traffic. The GE treatment, however, leaves afferent nerves intact. Comparisons were made of the contralateral right kidney sodium excretions before and after left kidney surgical and left GE denervation procedures. No significant differences were found. Consequently, these data in DOCA-treated miniature swine with established hypertension do not support the concept that renal efferent nerves influence contralateral renal efferent nerve activity.

Although in a normal animal the renal nerves appear to be of minimal importance in controlling basal renal hemodynamics or sodium excretion, numerous studies have indicated that they are important in the pathogenesis of various types of experimental hypertension. The results of this study in DOCA-hypertensive swine suggest that the renal nerves may also be important during the established phase of hypertension, exerting control over both renal hemodynamics and tubular sodium reabsorption.

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