Acute Renal Function in Chronically Sympathectomized Deoxycorticosterone Acetate-Treated Miniature Swine (43264)

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Abstract. We recently validated a swine model in which chronic treatment with 6-hydroxydopamine (6-OHDA) produced an effective sympathectomy. These sympathectomized swine demonstrated a significantly attenuated hypertensive response when treated with deoxycorticosterone acetate (DOCA). Because renal nerve activity is elevated and important in controlling renal function and blood pressure in the DOCA swine model, we wanted to study the effect of chronic sympathectomy on acute renal hemodynamics and tubular function. Kidney function was assessed in 14 DOCA-treated miniature swine, 8 of which were sympathectomized by chronic treatment with 6-OHDA, while 6 served as controls. Effective renal sympathectomy in this model has been previously confirmed by a significant reduction (97%) of norepinephrine in renal cortical tissue. When anesthetized, mean arterial pressure and renal blood flow were similar between the two groups. Glomerular filtration rate was lower by 43%, urine flow rate by 71%, sodium excretion by 66%, and potassium excretion by 48% in the 6-OHDA DOCA animals. All of these parameters were significantly different from the intact DOCA controls. These results indicate that anesthetized, chronically sympathectomized swine exhibit decreased renal excretory function. The changes in renal function may have been due to the development of a tubular or glomerular supersensitivity to circulating antinatriuretic factors, since the 6-OHDA group had a 28% greater pressor response to the α-agonist phenylephrine and a significantly greater fall in mean arterial pressure in response to α-blockade with prazosin when compared with the controls. These changes in renal function may also explain why the 6-OHDA animals demonstrated a slight increase in mean arterial pressure in response to DOCA. Because acute renal denervation in DOCA-treated swine produces a diuresis and natriuresis, this study affirms that there may be important functional differences in acutely versus chronically denervated kidneys for which the implications under normal physiologic conditions are unknown. [P.S.E.B.M. 1991, Vol 197]

Previous studies in deoxycorticosterone acetate (DOCA)-hypertensive miniature swine have demonstrated that peripheral sympathetic nerve activity is elevated and important in the development of the hypertensive state (1, 2). In this model, measured renal nerve activity is increased (3) and studies using acute surgical or pharmacologic renal denervation have shown that the nerves affect renal hemodynamics and augment tubular sodium reabsorption (4). More important, chronic surgical renal denervation, evaluated over a 3-week period, completely normalized blood pressure in miniature swine with established DOCA hypertension. This effect was associated with a leftward shift, or normalization, of the pressure-natriuresis curve (2).

Recently, we validated a swine model in which chronic treatment with 6-hydroxydopamine (6-OHDA) produced an effective, general sympathectomy (5). We have observed that the increase in blood pressure in response to DOCA was significantly reduced, but not completely eliminated, in these chronically sympathectomized animals (5). Because of the central role of the kidney in the control of blood pressure, we wanted to determine whether renal function in the 6-OHDA-treated animals was being altered in a different manner from what we have seen with either acute or chronic surgical renal denervation. A difference in kidney func-

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tion could possibly explain the inability of chemical sympathectomy to completely prevent an increase in blood pressure from occurring in response to DOCA. Consequently, a complete assessment of renal function was made in normal DOCA-hypertensive swine versus DOCA swine which had been chronically sympathectomized with 6-OHDA.

Materials and Methods

Fourteen male and female miniature swine were used for this study (Deerfield Mini-Pigs, Deerfield, NJ). Animals were approximately 6–12 months of age at the time of the study and ranged in weight from 10 to 33 kg. All animals were fed a swine ration which contained approximately 25 mEq of sodium/day. Water was provided *ad libitum.*

Of the 14 animals used, 8 were chronically treated with 6-OHDA hydrobromide (Aldrich, Milwaukee, WI) beginning on the first day after birth and continuing at weekly intervals (75 mg/kg ip) for the next 6 months as described previously (5). The remaining six animals served as controls. The validity of this protocol to produce an effective sympathectomy as assessed by both functional and biochemical means has been reported previously. In these animals, cortical norepinephrine content of the left kidney was 284 ± 41 ng/g in the control group versus 9 ± 4 ng/g in the 6-OHDA-treated group ($P < 0.05$) (5).

Approximately 5 weeks before the acute renal function studies, tygon catheters were placed in the left common carotid artery and left external jugular vein of each animal. The catheters were exteriorized on the dorsal surface of the neck. One week after this initial surgery, DOCA-impregnated silicone strips (200 mg/kg) were implanted subcutaneously in the left flank of each animal. All of the surgeries were conducted aseptically under halothane anesthesia. The effect of chronic treatment with 6-OHDA on the blood pressure response to DOCA in these animals has been reported previously. Briefly, conscious mean arterial pressure (MAP) was similar in the two groups of animals before DOCA implantation (125 ± 5, control; 116 ± 2 mm Hg, 6-OHDA). Three weeks after DOCA treatment, MAP had risen to 163 ± 6 mm Hg in the controls, but only to 135 ± 6 mm Hg in the 6-OHDA pigs (5).

One week before the acute renal function studies, the conscious MAP and heart rate (HR) responses to a bolus intravenous injection of 100 μg of phenylephrine hydrochloride (Sigma, St. Louis, MO) were determined in all of the animals. Each animal was placed in a hammock sling and a Statham pressure transducer was connected to the arterial catheter to measure MAP. Pulsatile and electronically averaged MAP were recorded on a Grass direct writing recorder. HR was determined from pulsatile blood pressure. Following the phenylephrine test, the MAP and HR responses to 0.5 mg/kg prazosin hydrochloride (Pfizer, Groton, CT) were determined in four of the 6-OHDA-treated animals and three of the controls. The drug was made fresh daily in a 5% glucose solution and was infused intravenously over a 5-min period. α-Receptor blockade was judged to be complete when the pressor response to phenylephrine (100 μg iv) was abolished. MAP and HR were followed for 20 min after the administration of prazosin.

At the time of the acute renal function studies, the animals were anesthetized with sodium pentobarbital (30 mg/kg iv), intubated, and mechanically ventilated. A Statham pressure transducer was connected to the arterial catheter to measure MAP. The jugular catheter was used to administer a prime (50 mg/kg) and sustaining dose of inulin in 0.9% saline a 1 ml/min to achieve a plasma inulin level of 0.2 mg/ml. The bladder was approached through a ventral midline incision and both ureters were cannulated. The left kidney was approached via a flank incision and an electromagnetic flow probe (Biotronex, Silver Spring, MD) or a Doppler flow probe (Crystal Biotech, Hollistion, MA) was placed around the left renal artery to measure renal blood flow (RBF). MAP and RBF were recorded continuously on a Grass direct writing recorder.

After a 45-min equilibration period, a 40- to 60-min urine collection was begun. Urine was collected separately from both kidneys and a blood sample was obtained at the midpoint of the collection period. After this collection period, a curved 25-gauge needle was inserted into the left renal artery distal to the flow probe and retrograde to the direction of blood flow in three of the 6-OHDA-treated animals and in three controls. The needle was attached with thin polyethylene tubing to an infusion pump (model 940; Harvard Apparatus, South Natick, MA). Saline was infused at 0.1 ml/min to maintain patency of the tubing. The MAP and RBF responses to separate intravenous and intrarenal injections of 50 μg of phenylephrine were recorded. At the end of the experiment, the flow probe was calibrated *in situ* by making timed collections of blood from a catheter placed in the renal artery distal to the probe.

Plasma and urine samples were analyzed for sodium and potassium via flame photometry and for inulin using the anthrone method. Glomerular filtration rate (GFR) was determined by inulin clearance. Renal vascular resistance was calculated as the quotient of MAP and RBF. Filtration fraction, expressed as a percentage, was calculated as the quotient of GFR and renal plasma flow. RBF and GFR were normalized for kidney weight.

Standard statistical techniques were used for the calculation of mean values and standard errors which are given in the text and figures. Data are presented for the left kidney only. For comparison between the intact DOCA and 6-OHDA DOCA group means, an unpaired

Student's t test was performed. A difference was considered significant if $P < 0.05$.

Results

Under pentobarbital anesthesia, MAP was similar in the control (123 ± 3 mm Hg) and 6-OHDA (124 ± 4 mm Hg) animals. As shown in Figure 1, RBF and renal vascular resistance also were similar in the control and 6-OHDA groups. GFR was 43% lower in the 6-OHDA animals when compared with the control group ($P < 0.05$, Fig. 1). Filtration fraction was similar in the 6-OHDA (11.3 ± 2.3%) and control (14.2 ± 1.9%) groups.

Urine flow rate (UV), sodium excretion ($U_{Na}V$), and potassium excretion were all significantly lower in the 6-OHDA group as shown in Figure 2. UV was lower by 71%, $U_{Na}V$ by 66% and potassium excretion by 48% when compared with the control group.

Fractional sodium excretion tended to be lower in the 6-OHDA animals (1.58 ± 0.57%) when compared with the control group (3.22 ± 0.63%), although this difference was not significant. Fractional potassium

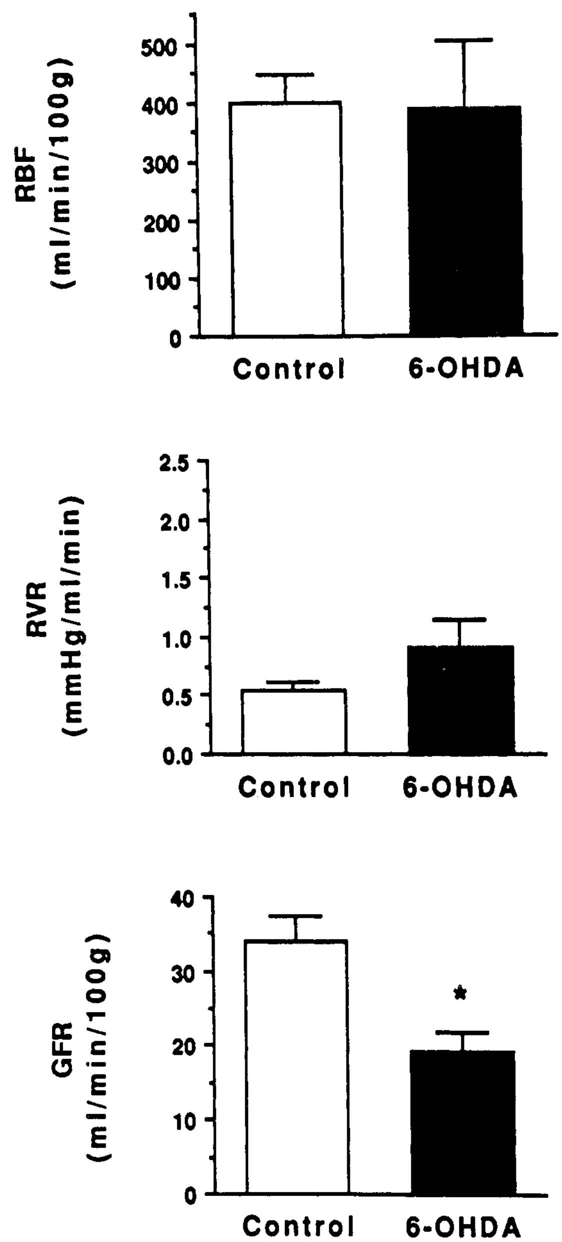

Figure 1. Renal blood flow (RBF), renal vascular resistance (RVR), and glomerular filtration rate (GFR) in six anesthetized control swine (open bars) and eight swine sympathectomized with 6-OHDA (solid bars). Both groups had been treated with DOCA 4 weeks before the study. * $P < 0.05$ versus control.

Figure 2. Urine flow rate (top graph) and absolute excretions of sodium (middle graph) and potassium (bottom graph) in six anesthetized control swine (open bars) and eight swine sympathectomized with 6-OHDA (solid bars). Both groups had been treated with DOCA 4 weeks before the study. * $P < 0.05$ versus control.

excretion was similar in the two groups (6-OHDA, 55.7 ± 11.1%; control, 58.5 ± 4.5%).

The conscious pressor response to phenylephrine was 28% greater in the 6-OHDA group ($P = 0.07$, Fig. 3). Figure 3 also shows a significantly greater fall in MAP in response to prazosin in the 6-OHDA group. While under anesthesia, intravenous phenylephrine increased blood pressure by 34 ± 2 mm Hg in the control group as compared with 59 ± 13 mm Hg in the 6-OHDA group ($P = 0.07$). Intrarenal phenylephrine decreased RBF by 32 ± 12% in the control animals and by 68 ± 22% in the 6-OHDA animals ($P > 0.05$).

Discussion

These data indicate that acute renal function in anesthetized DOCA-treated miniature swine was significantly altered by chronic sympathectomy. Although renal perfusion pressure and RBF were similar between the control and sympathectomized groups, UV, GFR, $U_{Na}V$, and potassium excretion were significantly lower in the sympathectomized animals. We have previously shown that the renal nerves are important during both the developmental and sustained phases of the hypertensive condition in DOCA-treated swine (1, 2, 5). Elevated renal nerve activity, which shifts the pressure-natriuresis relationship to the right, is responsible for the abnormal renal function exhibited by DOCA swine (4, 6). Therefore, chronic renal denervation should prevent the development of DOCA hypertension in swine. However, we recently found that chronic sympathectomy did not completely prevent a rise in MAP in response to DOCA (5). When renal function was examined in these sympathectomized animals in the present study, we found that their ability to excrete sodium and water was significantly reduced. These results are just the opposite of what we see with acute surgical or pharmacologic renal denervation, which produces a diuresis and natriuresis in anesthetized, DOCA-treated swine (4).

Assuming that tissue catecholamine levels adequately reflect sympathetic innervation of the kidney, the renal nerves could not have been involved in the sodium and water retention observed in this study, since renal norepinephrine content in these animals was reduced by 97%. Likewise, the renin-angiotensin system was probably not involved, because treatment with DOCA suppressed plasma renin activity to a similar extent in the 6-OHDA and control groups (by >78%) (5). It is possible that the kidneys of the control pigs were inadvertently denervated during the surgical placement of the renal blood flow probes, leading to an increase in sodium and water excretion during the

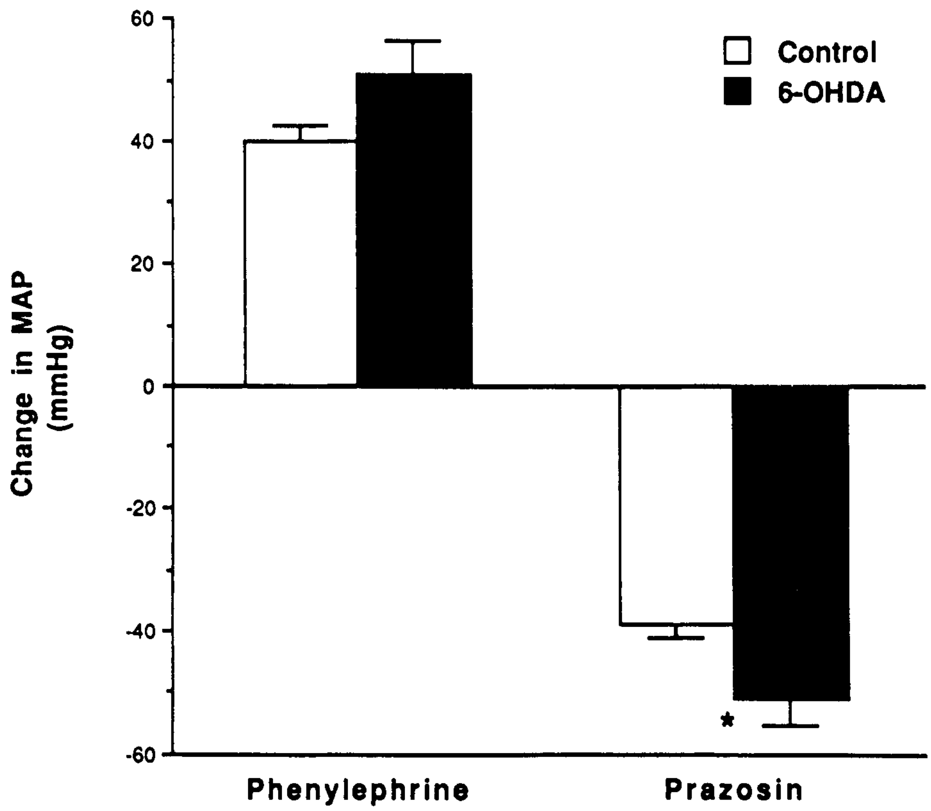

Figure 3. The change in mean arterial pressure (MAP) from conscious baseline values in response to the α_1-adrenoceptor agonist phenylephrine (100 μg iv) is shown on the left side and for the α_1-antagonist prazosin (0.5 mg/kg iv) on the right side. Swine sympathectomized with 6-OHDA are depicted by the solid bars ($n = 4$) and the control group is depicted by the open bars ($n = 3$). Both control and 6-OHDA groups had been treated with DOCA 3 weeks before these tests. * $P < 0.05$ versus control; for the phenylephrine test, $P = 0.07$.

experimental period. However, this would have been unlikely to occur consistently in all six of the control animals.

Renal hemodynamics did not appear to be responsible for the differences in sodium and water excretion since RBF, renal vascular resistance, MAP, and hence, renal perfusion pressure were the same for both groups during the acute studies. The fact that MAP was the same in the 6-OHDA and control groups was not surprising because we have previously reported that pentobarbital anesthesia decreases MAP to a normotensive level in 3- to 4-week DOCA-treated swine (7). We do not know if intrarenal hemodynamics were altered because renal blood flow distribution was not measured.

Other studies have shown that the adrenal medulla is not affected by 6-OHDA and it continues to produce and secrete catecholamines in an effort to compensate for the absence of an intact peripheral sympathetic nervous system (8). The development of supersensitivity to adrenergic agents is a well known effect of denervation (9). A vascular hyperresponsiveness to norepinephrine (NE) has been reported by a number of investigators following treatment with 6-OHDA (10–12). Yamada *et al.* (13) evaluated α- and β-adrenoceptor binding sites in the kidneys of rats sympathectomized with 6-OHDA. They found a reciprocal relationship between renal adrenoceptor density and the endogenous tissue NE concentration. Slotkin *et al.* (14) studied renal function in neonatally sympathectomized rats and found no difference in the number of β-receptor binding sites, but did report a significantly greater cyclic AMP response to stimulation of β-receptors.

As shown in Figure 3, the 6-OHDA animals in the present study displayed a 28% greater pressor response to the α-agonist phenylephrine ($P = 0.07$) and a 31% greater fall in MAP in response the α-antagonist prazosin ($P < 0.05$) when compared with the control group. Also, we have previously reported that venous NE is increased in DOCA hypertensive swine (1). These data would suggest that an increased sensitivity to circulating catecholamines could account for the antinatriuresis and antidiuresis exhibited by the sympathectomized animals via a tubular and/or a glomerular mechanism. However, we have no direct evidence of a change in the tubular response to NE (i.e., sodium transport) because the tests which we used to evaluate supersensitivity when the animals were conscious (phenylephrine, prazosin) assessed hemodynamic, and not tubular, responses.

The development of renal tubular supersensitivity to circulating catecholamines has been demonstrated by Szénási *et al.* (15) in dogs with chronically denervated kidneys. In their study, the chronically denervated animals tended to exhibit a lower UV, GFR, and $U_{Na}V$ and a higher RBF than dogs with innervated kidneys, although no significant differences were detected (15). α-Adrenoceptor blockade by intrarenal infusion of phenoxybenzamine produced a significant diuresis and natriuresis in the chronically denervated group, while the acutely denervated animals were unaffected (15). In addition, the authors found that the dose of NE needed to produce similar changes in all measured renal functional parameters was 50% lower in the chronically denervated dogs as compared with either the acutely denervated or intact groups (15).

The results of our study are also in agreement with those obtained by Krayacich *et al.* (16), in which the effect of NE infusion on renal function was evaluated in anesthetized rats with one intact and one chronically denervated kidney. Evidence was provided for both vascular and tubular supersensitivity to circulating NE that was within the physiologic range. When renal perfusion pressure was held constant, the denervated kidneys exhibited a decrease in absolute and fractional sodium excretion and urine flow in response to a low dose infusion of NE, while there were no changes in the innervated kidneys (16). The authors concluded that renal function may be altered in chronically denervated kidneys under certain conditions which would minimize the effect of renal denervation (16). The results of the present study, performed in anesthetized, chronically sympathectomized swine, support their hypothesis.

Interestingly, the 6-OHDA animals did not appear to be retaining salt and water in the conscious state during the 3 weeks of DOCA treatment, as evidenced by weight gains similar to those of the control animals and the lack of visible ascites or edema during the anesthetized experiments (5). Salt and water retention would have been expected based upon the results of the acute renal function experiments presented above. However, the changes in renal function that we observed in the anesthetized animals may not have been manifested fully in the conscious, relatively unstressed state. Hyperresponsiveness to circulating catecholamines, and possibly to other hormones such as vasopressin, may have become apparent only after induction of anesthesia and acute surgical stress.

We have shown that acute renal excretory function was significantly reduced by chronic sympathectomy in anesthetized, DOCA-treated miniature swine, whereas renal hemodynamics were relatively unaffected. These changes in renal function may explain the inability of chronic sympathectomy to completely prevent a DOCA-induced rise in MAP. The results of this study indicate that the natriuresis and diuresis commonly seen after acute renal denervation in this model may not persist and may reverse to an antinatriuresis and antidiuresis in a chronically denervated state. Thus, the beneficial effects of renal denervation to ameliorate salt and water retention may be lost with time as hyperresponsiveness to circulating catecholamines, and possibly other antinatriuretic factors, develops. Whether these same changes in renal function are evident in the conscious state, either during stressful or unstressful conditions, warrants further study.

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