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Renal Function in Two-Kidney Deoxycorticosterone Acetate-Hypertensive Yucatan Miniature Swine (41263)

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Abstract. To determine renal function in two-kidney deoxycorticosterone acetate (DOCA)-treated Yucatan miniature swine, eight animals were implanted with DOCA-treated silicone strips. After 3–4 weeks, mean arterial pressure (MAP) increased by approximately 32% to 148 ± 4 mm Hg. Eight sham-implanted control swine were also studied. Pentobarbital anesthesia decreased MAP in DOCA animals to 116 ± 6 mm Hg, a value similar to that seen in the anesthetized controls (112 ± 4 mm Hg). Renal blood flow and glomerular filtration rates were significantly higher in control as compared to DOCA animals ($P < 0.05$). Radioactive microsphere distribution to the outer cortex was also significantly decreased and inner cortical blood flow was significantly higher in the DOCA-treated animals ($P < 0.05$). Urine flow rates were similar between the two groups, whereas sodium excretion was lower and potassium excretion was higher in the DOCA-treated animals. These data indicate that DOCA-hypertensive Yucatan miniature swine appear to have alterations in renal function which were present when renal perfusion pressure was reduced to normal levels. The lowering of MAP by anesthesia suggests the involvement of a neurogenic component in the maintenance of hypertension.

Chronic deoxycorticosterone acetate (DOCA) treatment has been used in a number of studies to produce hypertension (1–3). However, administration of this mineralocorticoid to produce hypertension in rats or dogs has necessitated that the animal also be salt loaded and/or unilaterally nephrectomized. Recently, Terris and Simmonds (4), in a preliminary report, indicated that two-kidney DOCA-treated conscious Yucatan miniature swine, receiving 4.5 meq Na^+/kg/day, demonstrated a 40–50% increase in mean arterial pressure (MAP). These animals also exhibited hypernaturemia, hypokalemia, polyuria, polydipsia, hypochloremia, and metabolic alkalosis.

To date, renal function has not been examined in DOCA-treated Yucatan miniature swine. The purpose of this study was to determine if renal function is altered in this hypertensive animal model.

Methods. Sixteen male Yucatan miniature swine, 20–34 kg, were used for this study (Buckshire Corp., Perkasie, Pa.). Under sterile conditions and halothane anesthesia, eight swine were implanted with DOCA-impregnated silicone strips according to the procedure of Terris *et al.* (3). A catheter was also placed in the carotid artery and exteriorized on the animal's back to allow for the measurement of MAP. The control or normotensive group ($n = 8$) consisted of either nontreated or sham-silicone-implanted animals. Data from sham implanted and nonimplanted animals were similar, consequently their results were combined. All animals were fed a swine diet which contained approximately 50 meq of sodium per day. Water was provided *ad libitum*.

MAP was measured in conscious control and DOCA animals three to five times per week following the surgery. The arterial catheter was connected to a Statham transducer while the animals were suspended in a hammock-type sling (5). Arterial pressure was meaned electronically and recorded on a direct writing recorder. Seven of the eight DOCA-treated animals were studied 21–31 days postimplant. Data from one animal, studied after 8 days of DOCA treatment, were similar to those treated for the longer duration. Consequently, this animal's data were included in the results of the DOCA group. At the time of the study, the animals were anesthetized with ketamine hydrochloride (3.3 mg/kg) and sodium pentobar-

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bital (33 mg/kg). Animals were intubated and mechanically ventilated. Catheters were placed in the jugular vein, aortic arch, and abdominal aorta. The left kidney was approached via a flank incision. An electromagnetic flow probe (Biotronex) was placed around the left renal artery. A small catheter was placed in the left ureter. The bladder was approached via a midline incision and the right ureter was cannulated. Following the completion of surgery, animals were given a prime (62 mg/kg) and sustaining dose of inulin in 0.9% NaCl at 1 ml/min to achieve plasma inulin levels of 0.2 mg/ml. After a 45-min equilibration period, a 40- to 50-min control urine collection was begun. Blood samples were obtained at the midpoint of the control period. During the control period, approximately $1-2 \times 10^5$ cerium^{-141} -radiolabeled microspheres, 15 μm in diameter (New England Nuclear), were injected into the aortic arch. During microsphere injection a reference flow was obtained by a timed withdrawal of blood from the abdominal aortic catheter. Left renal blood flow and MAP were recorded continuously on a Grass direct writing recorder.

At the end of the experiment, the flow probe was calibrated *in situ* by making timed blood flow collections through a catheter placed in the renal artery distal to the probe. The left kidney was then removed and sectioned according to the method of Stein *et al.* (6) to determine intrarenal cortical blood flow distribution. Right kidney total blood flow was determined by the microsphere method (7). Reference blood samples and kidney sections were counted for cerium^{-141} using a Beckman gamma radiation counter.

Serum and urine samples were analyzed for sodium and potassium via flame photometry. Glomerular filtration rate (GFR) was determined by inulin clearance. Inulin concentration was measured by the anthrone method. Left renal vascular resistance was calculated as the quotient of MAP and RBF. Plasma renin activity (PRA) of samples obtained during the control period was determined by radioimmunoassay for angiotensin I (New England Nuclear).

Results are expressed as mean ± SEM. Values from control and DOCA-treated animals were evaluated using an unpaired Student's t test. All differences were considered significant if $P < 0.05$.

Results. In the conscious DOCA-treated animals, MAP rose from approximately 110–115 to 148 ± 4 mm Hg. After 3–4 weeks of DOCA treatment, pentobarbital anesthesia caused a reduction in MAP in the DOCA pigs; consequently, under anesthesia, MAP was similar in control and DOCA animals. (Control = 112 ± 4 mm Hg; DOCA = 116 ± 6 mm Hg). Left renal blood flow was 26% less in the DOCA animals as compared to the controls (5.1 ± 0.5 ml/g/min control; 3.8 ± .3 ml/g/min DOCA) ($P < 0.05$). Right kidney blood flow was also approximately 21% lower in the DOCA animals ($P < 0.05$). Left renal vascular resistance was elevated in the DOCA animals (23.7 ± 3.3 mm Hg/ml/min/g control; 32.6 ± 3.9 mm Hg/ml/min/g DOCA). This difference, however, was not significant. Fractional and absolute microsphere distributions to cortical sections C_1-C_4 are shown in Fig. 1. Control animals showed a left kidney outer (C_1) to inner (C_4) cortical distribution gradient with outer cortical distribution being greater than inner. In the DOCA animals there was essentially no cortical microsphere distribution gradient between zones C_1 through C_4. Microsphere mixing appeared to be adequate because of only a 2.8% variability between counts obtained from sections from the left vs right kidneys. Left and right kidney urine flow

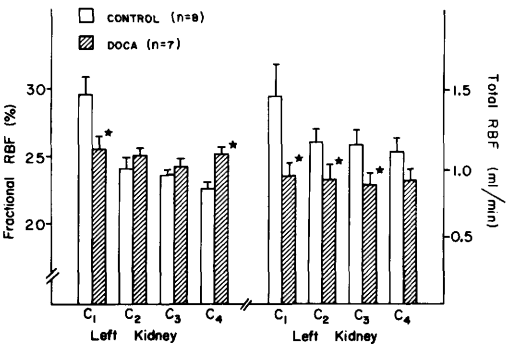

FIG. 1. Left kidney fractional and absolute intrarenal cortical microsphere distribution. C_1 = outermost cortex; C_4 = innermost cortex. Asterisks represent $P < 0.05$ versus control. Values are mean ± SEM.

rates were similar in control and DOCA animals (Table I).

Whole-animal GFR was significantly lower in the DOCA group (97.5 ± 7.5 ml/min/100 g control; 76.0 ± 7.0 ml/min/100 g DOCA ($P < 0.05$). Left and right kidney GFR (Fig. 2) were approximately 22% lower in the DOCA animals as compared to the controls. The difference was significant only for the left kidneys. Serum sodium values for the control and DOCA pigs were 138 ± 2 and 142 ± 2 meq/liter, respectively. Serum potassium was significantly lower in the DOCA animals (5.8 ± 0.5 meq/liter control; 4.1 ± 0.3 meq/liter DOCA ($P < 0.05$). Plasma renin activity was 0.26 ± 0.08 ngAI/ml/hr in the control animals, but ranged from nonmeasurable levels to 0.04 ngAI/ml/hr in the DOCA animals with a mean value of 0.03 ± 0.01 ngAI/ml/hr in the DOCA pigs. Electrolyte excretion rates are shown in Table I. Left and right sodium excretions were lower in DOCA animals as compared to controls. The difference, however, was only significant for the right kidneys ($P < 0.05$). Total sodium excretion in the DOCA group was significantly lower than control excretion (84.1 ± 25.3 μeq/min control; 32.9 ± 8.8 μeq/min DOCA) ($P < 0.05$). Urinary potassium excretion was 31 and 18% higher in the left and right kidneys, respectively, of the DOCA animals (Table I). Total potassium excretion was 72.8 ± 5.9 μeq/min in the control group and 88.2 ± 16.1 μeq/min in the DOCA group. These differences in individual kidney and total potassium excretion were not significant between the DOCA and control animals. Fractional sodium excretion (Table I) was less in both kidneys of the DOCA animals as compared to controls, with the difference between the right kidneys being significant ($P < 0.05$). Whole animal fractional sodium excretion was 0.73 ± 0.25 and 0.22 ± 0.06% in the control and DOCA animals, respectively. This difference was significant ($P < 0.05$).

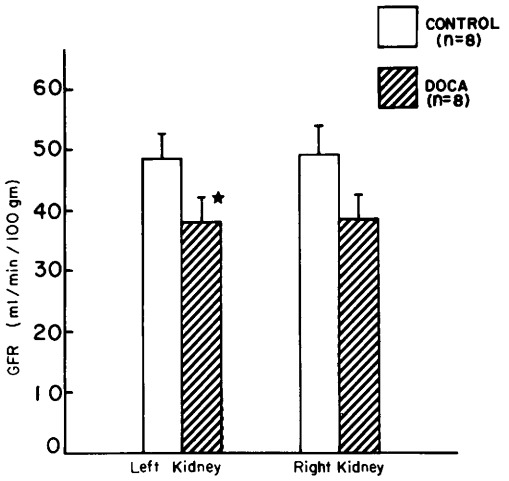

FIG. 2. Glomerular filtration rate (GFR) in control and DOCA animals. Values are mean ± SEM (*$P < 0.05$).

Discussion. The results of this study agree with the preliminary reports of Terris and associates (4, 8) that two-kidney Yucatan miniature swine receiving 300–400 meq Na^+/day develop hypertension following DOCA treatment. These data also indicate that DOCA treatment will cause hypertension even if the animals are maintained on a relatively low sodium diet (50–75 meq/day).

An unexpected finding was that sodium pentobarbital caused a reduction in MAP in the DOCA pigs. Previous measurements of MAP in DOCA-treated Yucatan miniature

TABLE I. URINE FLOW RATE, SODIUM EXCRETION ($U_{Na}V$), POTASSIUM EXCRETION (U_KV), AND FRACTIONAL SODIUM EXCRETION (FE_{Na}) IN CONTROL AND DOCA ANIMALS

	Left kidney		Right kidney	
	Control	DOCA	Control	DOCA
Urine flow (ml/min)	0.36 ± 0.07	0.36 ± 0.06	0.50 ± 0.11	0.45 ± 0.08
$U_{Na}V$ (μeq/min)	33.0 ± 10.0	19.9 ± 7.3	51.0 ± 15.8	14.2 ± 2.6*
U_KV (μeq/min)	33.1 ± 2.6	43.5 ± 8.1	39.7 ± 3.7	46.8 ± 9.0
FE_{Na} (%)	0.57 ± 0.18	0.28 ± 0.10	0.89 ± 0.32	0.19 ± 0.03*

Note. $n = 8$.
* $P < 0.05$.

swine have been made in conscious animals (4). Anesthesia did not decrease MAP in the control animals. We have also observed that halothane or ketamine decreases MAP in DOCA animals. Numerous studies have indicated that in the rat DOCA hypertension is associated with increased sympathetic nervous system activity (1, 9, 10). Since these anesthetic agents are known to depress sympathetic nerve activity (11, 12), these results reinforce the possibility that sympathetic nervous activity may also be increased in the DOCA-treated Yucatan miniature swine. Studies using surgical and pharmacological interventions to block sympathetic nervous activity are required to evaluate the role of the sympathetic system in this hypertensive model.

DOCA-treated animals had significantly lower RBF and GRF when compared to control animals. These differences in renal hemodynamics were present despite the fact that MAP or renal perfusion pressure was similar for both groups. It has been reported that in the DOCA-salt-hypertensive rat, the renal vascular bed may receive a disproportionate amount of sympathetic nerve activity which results in a greater vasoconstriction as compared to other vascular beds (1). Elevated renal sympathetic adrenergic activity or an increase in responsiveness to sympathetic stimuli may explain the increased renal vascular resistance observed in these DOCA-treated animals. Since we only measured RBF and GFR at normal renal perfusion pressures, we do not know if these parameters would autoregulate in response to the elevated pressure (i.e., remain the same) or whether they would increase to levels comparable to what was seen in the controls.

Urine flow rates in the DOCA animals were similar to that seen for the controls. This contrasts the polyuria observed in DOCA-treated conscious Yucatan miniature swine (4). This difference may have been due to the differences in renal perfusion pressure, or the fact that these animals were receiving a relatively low sodium diet as compared to previous studies on Yucatan miniature swine.

The sodium retention observed in the DOCA-treated animals could have been due to renal vasoconstriction and/or increased tubular sodium reabsorption. Both mechanisms may be mediated by increased sympathetic activity (13). The fractional sodium excretions (Table I) suggest that the sodium retention seen in the DOCA-treated animals was partially due to enhanced sodium reabsorption.

This study demonstrates that in the two-kidney DOCA-treated Yucatan miniature swine, the hypertension that develops is abolished by barbiturate anesthesia. At control renal perfusion pressures DOCA-treated animals demonstrate augmented renal vasoconstriction and sodium retention. These differences in renal function between control and DOCA pigs which are observed after 3–4 weeks of DOCA treatment may become even more pronounced if DOCA treatment is continued for a longer period of time. The mechanisms responsible for these changes remain to be elucidated.

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