Significance of Sodium, Sympathetic Innervation, and Central Adrenergic Structures on Renal Vascular Responsiveness in DOCA-Treated Rats

KATHLEEN H. BERECEK, ROBERT D. MURRAY, AND FRANZ GROSS

SUMMARY The effects of sodium, sympathetic innervation, and central adrenergic structures on the development of changes in renal vascular reactivity were studied in unilaterally nephrectomized rats treated with a single implant of deoxycorticosterone acetate (DOCA; 100 mg/kg). Vascular reactivity to norepinephrine (NE) and vasopressin (ADH) was assessed in isolated kidneys perfused with a synthetic medium. Influence of sodium was determined by placing DOCA-treated rats on high, normal, and low sodium intakes. Neural influence was studied by means of local denervation of the renal artery and by intravenous (iv) and intraventricular (ivt) administration of 6-hydroxydopamine (6-OHDA). Marked changes in renal vascular reactivity in DOCA-treated rats were already apparent prior to the rise in blood pressure. Dose-response curves for NE and ADH showed parallel leftward shifts and decreased threshold doses. Normal sodium intake, local denervation, and peripheral sympathectomy had no effect on the development of these vascular changes in DOCA-treated rats. However, sodium deficiency and ivt administration of 6-OHDA totally prevented development of enhanced vascular reactivity. These results imply that increased vascular reactivity is a major factor in the development of DOCA-hypertension. Circ Res 47: 675-683, 1980

EXPERIMENTAL findings have supported the participation of the sympathetic nervous system in the initiation of hypertension induced by deoxycorticosterone acetate (DOCA) and salt (deChamplain, 1973; Haeusler et al., 1972). In the DOCA-hypertensive pig, an enhanced vascular reactivity was observed prior to a significant rise in arterial pressure (Berecek and Bohr, 1978). Recently, we have demonstrated in rats that an increase in renal vascular reactivity to pressor agents occurred 4 days after DOCA implantation, well before a rise in arterial pressure (Berecek et al., 1980). These data suggest that increased vascular reactivity, per se, is a pathogenic factor in the development of DOCA and salt hypertension.

The purpose of the present study was to assess the interrelationship of sympathetic activity, sodium balance, and vascular reactivity in the development of DOCA hypertension.

Methods

Experimental Animals

Male Sprague-Dawley rats (200-270 g body weight) were used. The rats were housed individually in plastic cages. The left kidney was removed under ether anesthesia 5-7 days before the beginning of experimental manipulations. Subsequently, the experimental rats received subcutaneous implants of Silastic strips (silicone rubber, Dow-Corning Co.) impregnated with deoxycorticosterone acetate (DOCA, Sigma Chemical Co.) at a dose of 100 mg/kg. Control rats received Silastic implants without DOCA; they also were unilaterally nephrectomized. In all groups, perfusion of the right kidney was performed 3-4 days after DOCA or Silastic implantation. The influence of sodium on the appearance of changes in vascular reactivity was assessed by placing separate groups of experimental and control rats on sodium-deficient, normal sodium, and high sodium intake. The effect of the peripheral sympathetic system was estimated by local denervation of the kidney and "chemical sympathectomy" with intravenous (iv) administration of 6-hydroxydopamine (6-OHDA) and that of the central nervous system by intraventricular (ivt) administration of 6-OHDA. All groups of rats except those of normal or sodium-deficient intakes received a 0.9% NaCl + 0.2% KCl solution to drink. Systolic blood pressures were recorded by tail-cuff plethysmography (W+W electronic blood pressure recorder, W+W Electronics) under light ether anesthesia two times prior to the implantation of DOCA or Silastic and on two occasions thereafter.

Sodium Intake

Groups placed on a sodium-deficient diet received a modified Hartroft-Eisenstein diet (Mohring and Möhring, 1972) and distilled water to drink.
Groups on normal salt intake were fed a standard laboratory pellet chow (ssnif) containing sodium (100 mmol/kg), potassium (210 mmol/kg), and tap water to drink. Rats on high sodium intake had the standard laboratory chow and 0.9% NaCl to drink. The various diets were administered 1 day prior to the implantation of DOCA or Silastic.

Renal Denervation

On the occasion of the left nephrectomy, the artery to the right kidney was exposed and painted with a 10% phenol solution, stroking the artery 10 times each on the two opposing sides. One week after the denervation, the DOCA or Silastic strips were implanted and all rats were given 0.9% NaCl to drink.

Peripheral Sympathectomy

Two doses of 6-OHDA (2,4,5-trihydroxyphenyl-ethylamine hydrobromide; Sigma Chemical Co.) of 50 mg/kg each were given iv within 24 hours. The 6-OHDA was dissolved in a 1% solution of ascorbic acid in normal saline saturated with nitrogen. Two additional doses of 6-OHDA of 100 mg/kg each were given 1 week later. Five days after the first series of injections of 6-OHDA, the rats received DOCA or Silastic plus 0.9% NaCl. Renal perfusions were carried out 3 or 4 days postimplantation or 1–2 days, respectively, after the second series of injections of 6-OHDA.

Central Denervation

Central adrenergic structures were destroyed by the ivt administration of 6-OHDA. A cannula was implanted stereotaxically into the right lateral cerebral ventricle under pentobarbital anesthesia (Nembutal, Abbott Laboratories) 1.7 mm anterior to the intraaural line, 1.3 mm left of the sagittal suture, and 5.0 mm ventral from the surface of the skull. Seven days after the cannula had been placed, the rats were unilaterally nephrectomized. Five days later, administration of 6-OHDA was started. The rats received one ivt injection (250 µg/rat, 15 µl volume) followed by a second injection of the same dose 48 hours later. Seven to 14 days after the last ivt injection, DOCA was implanted and 0.9% NaCl was given to drink.

Isolation and Perfusion of the Kidney

After anesthesia with sodium pentobarbital (50 mg/kg, ip) the kidney was surgically isolated and perfused with modifications of the technique previously described by Hofbauer et al. (1973). The right kidney was perfused through a catheter placed into the distal aorta and advanced to the origin of the right renal artery. The kidneys were perfused at constant flow, using a Harvard peristaltic perfusion pump which was pressure independent to pressures in excess of 200 mm Hg. The perfusate was a modified Krebs-Henseleit solution containing Ficoll 70 (Pharmacia AB), 35 g/liter. The pH of the perfusate was 7.4; it was maintained at a temperature of 37°C and aerated with a mixture of 95% O2 and 5% CO2. Perfusion pressure, measured from the side arm of the aortic cannula (transducer-Statham P 23 Db) and perfusate flow, measured from the renal vein catheter (drop counter) were recorded continuously on a Sanborn polygraph (Hewlett-Packard, model 7702B). An equilibration period of 60 minutes was allowed before the experimental protocol was started.

Experimental Protocol

Vasoconstrictors employed for the reactivity studies were the following: norepinephrine (NE, Arterenol; Hoechst), and vasopressin (ADH, Pitressin, Parke-Davis). Perfusion flow was approximately 5.5 ml/g per min. At this flow, the renal vascular bed was considered to be tonic, as bolus injections of papaverine (Roche) into the arterial bed produced no further fall in perfusion pressure. Baseline perfusion pressure was stable for the duration of the experiment (140–160 minutes). After the equilibration period, cumulative dose-response curves to vasoconstrictors were obtained in the following order: NE and ADH. A period of 20 minutes was allowed between the administration of the different drugs. Doses of the drugs were applied intra-arterially in bolus injections of 10µl volumes from subthreshold to maximum doses.

Statistical Methods

All values presented in the text and in the figures are means ± se. The effect of sodium intake on changes in vascular reactivity was determined by one-way analysis of variance (ANOVA) among the groups of DOCA-treated rats receiving normal, high, and sodium-deficient diets. A separate ANOVA was run among the groups of control rats on the three sodium regimens. Similarly, the influence of the peripheral and the central nervous system on vascular reactivity was assessed by ANOVA; DOCA and control rats were analyzed separately. In all cases, when a significant (P < 0.05) F-ratio was obtained, the Newman-Keuls test was used to determine which of the groups differed significantly from the others. Comparisons between similarly treated DOCA and control rats were made using the Student's t-test. Finally, paired t-tests were used to evaluate systolic blood pressure before and after the implantation of DOCA or silastic.

Results

At an average of 3.5 days after DOCA implantation + 0.9% NaCl, the systolic blood pressure of the rats prepared in the standard manner had not increased significantly (Table 1). Moreover, renal vascular resistance was similar to that in kidneys from age and sex-matched control rats (Table 2). However, renal vascular reactivity to NE (Fig. 1b) and
Table 1: Characteristics of the DOCA at Perfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (g)</th>
<th>Pre-Doca</th>
<th>Post-Doca</th>
<th>Renal perfusion pressure (mm Hg)</th>
<th>Renal perfusate flow (ml/g per min)</th>
<th>Renal vascular resistance (mm Hg/ml per g per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOCA-high sodium (n: 8)</td>
<td>200.0 ± 0.98</td>
<td>110.6 ± 5.2</td>
<td>111.5 ± 2.9</td>
<td>38.5 ± 1.5</td>
<td>5.5 ± 0.11</td>
<td>6.80 ± 0.22</td>
</tr>
<tr>
<td>DOCA-normal sodium (n: 6)</td>
<td>264.3 ± 12.5</td>
<td>110.0 ± 5.4</td>
<td>102.3 ± 7.6</td>
<td>36.3 ± 1.4</td>
<td>5.5 ± 0.14</td>
<td>6.50 ± 0.24</td>
</tr>
<tr>
<td>DOCA-sodium deficient (n: 6)</td>
<td>204.2 ± 11.9</td>
<td>105.7 ± 5.4</td>
<td>105.4 ± 2.6</td>
<td>32.7 ± 1.2</td>
<td>5.5 ± 0.11</td>
<td>5.88 ± 0.14</td>
</tr>
<tr>
<td>DOCA + renal denervation</td>
<td>203.0 ± 9.6</td>
<td>112.0 ± 4.7</td>
<td>111.5 ± 3.9</td>
<td>38.3 ± 0.8</td>
<td>5.6 ± 0.06</td>
<td>6.80 ± 0.14</td>
</tr>
<tr>
<td>DOCA + iv 6-OHDA (n: 6)</td>
<td>208.7 ± 5.5</td>
<td>113.1 ± 4.0</td>
<td>110.0 ± 4.3</td>
<td>38.0 ± 1.0</td>
<td>5.6 ± 0.07</td>
<td>6.56 ± 0.34</td>
</tr>
<tr>
<td>DOCA + ivt (n: 9)</td>
<td>222.1 ± 6.4</td>
<td>114.7 ± 3.9</td>
<td>113.7 ± 3.1</td>
<td>37.0 ± 1.1</td>
<td>5.6 ± 0.08</td>
<td>6.56 ± 0.22</td>
</tr>
</tbody>
</table>

Responses are expressed as group mean ± se.
* 3.5 days post-DOCA implantation.
† Determined by tail-cuff plethysmography under light ether anesthesia.
‡ Significantly different from the other two salt groups (P < 0.05).

ADH (Fig. 2b) was enhanced as compared to control rats on a high sodium diet. Dose-response curves from kidneys of prehypertensive DOCA rats showed parallel leftward shifts and decreased thresholds.

Effect of Sodium Intake

Short-term changes in sodium intake in the control rats had little effect on systolic blood pressure, renal perfusion pressure, and vascular resistance, with the exception that rats receiving a sodium-deficient diet had slightly (P > 0.05) lower renal perfusion pressures and vascular resistances at flows comparable to those used for control rats on normal and high sodium intakes (Table 2). Furthermore, renal vascular reactivity to NE (Fig. 1a) and ADH (Fig. 2a) remained unaffected (P > 0.10). In contrast, in DOCA-treated rats, a sodium-deficient intake resulted in a concomitant decrease (P < 0.05) in renal perfusion pressure and vascular resistance (Table 1). Moreover, removal of sodium from the diet prevented the development of enhanced vascular reactivity to NE and ADH (Figs. 1b and 2b). Responses to all doses of NE with the exception of the two largest doses (10^-7-10^-6) were significantly less (P < 0.01) in renal vascular beds of DOCA rats on sodium-deficient diets than in DOCA rats on normal or high sodium intakes. Responses to ADH were also significantly reduced (5 x 10^-11-1.5 x 10^-10 g; P < 0.05; 5 x 10^-10-1.5 x 10^-9 g, P < 0.01) in DOCA rats on sodium-deficient diets as compared to DOCA rats on normal or high sodium intakes. Vascular reactivity to NE in DOCA rats on sodium-deficient diet was lower (3 x 10^-8-3 x 10^-7 g, P < 0.05) than that of kidneys from control (Silastic) rats (Fig. 1b), whereas the response to ADH was similar (P > 0.05) in the two groups.

Local Denervation of the Renal Artery

Local denervation of the renal artery had no effect on systolic blood pressure or perfusion characteristics in either the control rats (Table 2) or the DOCA-treated rats (Table 1). A denervation supersensitivity to exogenous NE (Fig. 3a) was seen in both control (3 x 10^-11-10^-9 g, P < 0.05; 3 x 10^-9-10^-7, P < 0.01; 10^-6 g < 0.05) and DOCA-treated rats (3 x 10^-10-10^-9 g, P < 0.01). However, vascular reactivity to NE in the denervated kidneys of DOCA rats was still significantly (P < 0.01) greater than that in denervated kidneys from control rats. No effect of denervation on renal vascular reactivity
Peripheral Sympathectomy

Peripheral sympathectomy with iv 6-OHDA had no significant effect on systolic blood pressure or renal perfusion characteristics in either control rats (Table 2) or in DOCA rats (Table 1). Both groups developed a denervation supersensitivity to exogenous NE (Fig. 4a). Responses of kidneys from control rats treated with iv 6-OHDA were greater \( (P < 0.05) \) than those of intact, control rats at doses of NE from \( 10^{-8} \) to \( 3 \times 10^{-7} \) g, and similar observations were made in kidneys from sympathectomized and intact DOCA rats. Responses of DOCA rats treated with 6-OHDA were significantly greater \( (P < 0.01) \) than those of intact DOCA rats at doses of NE from \( 10^{-8} \) to \( 10^{-7} \) g. Nevertheless, vascular reactivity to NE in kidneys from DOCA rats treated with iv 6-OHDA was greater \( (P < 0.01) \) than that in kidneys from control rats treated with iv 6-OHDA. No effect on reactivity to ADH was obtained in control rats, but reactivity to ADH was enhanced in renal vascular beds of DOCA rats given iv 6-OHDA (Fig. 4b). Responses of the sympathectomized DOCA rats were greater \( (P < 0.05) \) than intact DOCA rats at doses of ADH from \( 5 \times 10^{-10} \) to \( 0.5 \times 10^{-8} \) g.

Alteration in Central Adrenergic Structures

Intraventricular administration of 6-OHDA in DOCA rats was often found to produce aphagia and adipsia. Many rats treated in this manner lost weight and failed to survive. During the time of injections of 6-OHDA and for 2 days following the last injection, rats thrived better when the water intake was forced with an eye dropper and the rats were fed a diet to which sugar was added. The normal pellet chow was ground and water and table sugar were added to form a paste. This diet was more readily eaten than the pellet chow. In spite of special attention, some of the rats did not resume a normal eating and drinking pattern. Rats were selected for experimentation only when they had not lost weight and were eating and drinking freely.

Figure 1. Dose-response curves to norepinephrine in isolated perfused kidneys from control (Silastic) rats (a) and DOCA-treated rats (b) on different sodium intakes. Response (ordinate) is given as the increase in renal vascular resistance (mm Hg/ml per g per min) which was calculated as the quotient of the recorded increase in perfusion pressure (mm Hg) divided by the constant perfusate flow (ml/g per min) from maximal vasodilation. Responses are expressed as mean ± SE. Statistics are indicated in the text (see Results). n: number of rats in each group; abscissae: dose of norepinephrine (g/10 μl injection volume).
Destruction of central adrenergic structures had no effect on systolic blood pressure or renal perfusion pressure and vascular resistance (Table 1). However, the development of enhanced renal vascular reactivity to NE and ADH (Fig. 5, a and b) in DOCA rats was totally prevented. Responses to NE and ADH in DOCA rats treated with ivt 6-OHDA were significantly less ($P < 0.01$) than responses of kidneys from intact DOCA rats (NE: $10^{-11} - 3 \times 10^{-7}$g; ADH: $5 \times 10^{-11} - 1.5 \times 10^{-8}$g) and indistinguishable from responses obtained in kidneys from control rats.

**Discussion**

In this study we have assessed the effect of sodium, peripheral sympathetic control, and central adrenergic structures on the development of early vascular changes after DOCA implantation. Whereas an intact peripheral sympathetic system is not a necessary requirement for the development of enhanced reactivity, the presence of sodium and intact central adrenergic structures have profound effects on the reactivity of the renal vascular bed.

**Sodium**

The development of DOCA hypertension (de Champlain, 1973) and enhanced vascular reactivity (Abboud, 1974; and Schomig, et al., 1976) depend upon the presence of sodium. Previous observations that a low sodium diet renders DOCA-treated rats normotensive (de Champlain, 1973) and our finding that increased reactivity was totally prevented in rats on sodium-deficient diets lend strong support to the main role of sodium. An increased concentration of sodium in vascular smooth muscle cells might cause an enhanced reactivity to vasoactive substances in various ways: (1) by decreasing the resting membrane potential (Bulbring, 1962); (2) by producing an alteration in the sequestration of calcium (Sitrin and Bohr, 1971) or transport of calcium out of the cell (Blaustein, 1977); (3) by decreasing the re-uptake of norepinephrine by nerve endings.

**Figure 2** Dose-response curves of the same kidneys as in Figure 1 to vasopressin. Ordinate as in Figure 1. Statistics are indicated in the text (see Results); abscissae: dose of vasopressin (g/10 μl injection volume).
in the vascular wall (White, 1976); and (4) by increasing amino acid transport and thereby the protein-synthesizing activity of the vascular smooth muscle cell (Heinz et al., 1975). Both isotopic flux (Jones and Hart, 1975) and ion exchange studies (Friedman and Nakashima, 1978) present evidence for an increase in the passive permeability to sodium in arteries from DOCA-treated rats. An alteration of active transport of ions across the smooth muscle cells has also been discussed, but it has not yet been resolved whether Na\textsuperscript{+}–K\textsuperscript{+} ATPase activity is increased (Friedman and Nakashima, 1978) or decreased (Pamnani et al., 1978).

Peripheral Sympathectomy

The pattern of intravenous administration of 6-OHDA which was used (Finch et al., 1973a) and our findings of a denervation supersensitivity to exogenous NE give evidence for a marked functional sympathectomy of the vascular system. No effect on the early development of increased renal vascular reactivity was observed. Both control and DOCA animals developed a denervation supersensitivity to NE; however, the reactivity of the DOCA rats was still significantly greater than that of control rats. Response to ADH was slightly enhanced in DOCA rats which had received 6-OHDA as compared to untreated DOCA rats, whereas 6-OHDA had no effect on the responsiveness of kidneys from control rats. Similar results were obtained when the renal artery was denervated with phenol. Histological examination of tissue slices from kidneys denervated in this manner showed that there was no catecholamine fluorescence at 6 weeks postdenervation. It has also been shown that an intact sympathetic nervous system was not a requirement for the development of an increased responsiveness to NE or an increased potassium turnover in aortae from DOCA hypertensive rats (Jones et al., 1977).

There has been much indirect evidence that the onset of hypertension with DOCA and salt may be related to an alteration in sympathetic nerve function (de Champlain et al., 1969, 1976). However, results from studies using 6-OHDA to produce

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**Figure 3** Dose-response curves to norepinephrine (a) and vasopressin (b) in isolated perfused kidneys from control (○) and DOCA rats (▲) denervated with phenol as compared to intact control (○) and DOCA rats (▲). Ordinates as in Figure 1. Statistics are indicated in the text (see Results), abscissae: (a) dose of norepinephrine (g/10 μl injection volume) (b) dose of vasopressin (g/10 μl injection volume).
chemical sympathectomy have been conflicting. Mueller and Thoenen (1970) found that chemical sympathectomy delayed the onset of DOCA hypertension but failed to prevent its development, whereas, in other studies, 6-OHDA failed to alter either the onset or maintenance of DOCA hypertension (Finch and Leach, 1970; Clark et al., 1970). The main objections against the use of 6-OHDA have been that destruction of nerves is not permanent, that the adrenal gland is not affected, and that innervation of the vasculature is relatively resistant to the destructive effects of 6-OHDA and regeneration rapidly occurs (Kostrzewa and Jacobowitz, 1974). When animals are sympathectomized from birth with 6-OHDA, a widespread permanent destruction of both peripheral and central sympathetic neurons occurs. Nonetheless, Finch et al. (1973b) found that this administration slightly retarded but did not prevent development of DOCA hypertension. Furthermore, recent studies have shown that neonatal sympathectomy potentiated DOCA-salt induced hypertension (Proovost and De Jong, 1978). Our findings and those of others suggest that neither the onset of DOCA hypertension nor the development of increased vascular reactivity are causally related to peripheral sympathetic nerve function.

Central Adrenergic Structures

In contrast to the lack of effect of peripheral administration of 6-OHDA on vascular reactivity, injection of this substance into the lateral ventricles of rats prior to the implantation of DOCA totally inhibited the development of enhanced vascular reactivity. Thus, the dose-response curves obtained from DOCA rats were virtually identical to those from controls. Haeusler et al. (1972), using a similar protocol, found that ivt 6-OHDA given prior to DOCA totally prevented the development of hypertension and the development of increased vascular reactivity in the perfused mesenteric preparation. Administration of 6-OHDA in rats with established DOCA hypertension had no effect on blood pressure despite considerable destruction of central adrenergic structures.

FIGURE 4 Dose-response curves to norepinephrine (a) and vasopressin (b) in isolated perfused kidneys from control (●) and DOCA rats (▲) treated with iv 6-OHDA as compared to intact control (○) and DOCA rats (●). Ordinates as in Figure 1 and abscissae as in Figure 3. Statistics are indicated in the text (see Results).
Figure 5  Dose-response curves to norepinephrine (a) and vasopressin (b) in DOCA rats (●) treated with ivt 6-OHDA as compared to intact DOCA rats (●) and control rats (○). Ordinates as in Figure 1 and abscissae as in Figure 3.

6-OHDA injected into the lateral ventricle distributes widely throughout the central nervous system affecting NE and DA neurons in all parts of the brain and spinal cord, and there have been few studies attempting to localize the specific central adrenergic systems involved in hypertension. Recently, it was shown that intraspinal administration of 6-OHDA, which produced virtually complete loss of NE only in the cord, had no effect on the development of DOCA hypertension, making it unlikely that NE projections in the cord are essential (Kubo and Hashimoto, 1979).

Although it has been speculated previously that a central mechanism participates in the development of hypertension by increasing peripheral sympathetic outflow, it could also be related to processes which are endocrine in nature and governed by central adrenergic neurons. Recent experiments on DOCA-hypertensive rats suggest that ADH is increased in this model (Möhring, 1978). Furthermore, electrolytic destruction in the anteroventral
Vasopressin has been found to be a potent vasoconstrictor of resistance vessels in the physiological range (Altura and Altura, 1977) and can potentiate catecholamine-induced contraction in the microvasculature (Bartelstone and Nasmyth, 1965). From our findings, it is evident that the development of DOCA hypertension is related to the interaction of several factors. Early central stimulation of peripheral sympathetic activity and/or release of hormones from the brain in the presence of sodium may lead to an increase in vascular reactivity and resistance which, in turn, underlie the rise in arterial pressure. The observation that enhanced vascular reactivity occurs prior to the rise in arterial pressure and that sodium deficiency and destruction of central adrenergic structure known to prevent the development of hypertension also prevent the development of these early vascular changes suggest that the vascular smooth muscle cell is the common pathway whereby sodium in combination with a central mechanism produces the rise in arterial pressure.

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