Hemodynamic, Endocrine, and Electrolyte Changes During Sodium Restriction in DOCA Hypertensive Pigs

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SUMMARY Effects of a low sodium diet on hemodynamic, endocrine, and electrolyte variables were studied in nine DOCA-hypertensive and three control pigs. Sodium intake was reduced from about 200 mEq/24 hr to 20 mEq/24 hr. Low sodium caused the blood pressure (BP) of five pigs to return to within 5% of normal levels (responders); in four others (nonresponders), BP remained elevated. Cardiac outputs were the same in all DOCA-treated pigs and not different from pre-low sodium values. Therefore, BP fell in the responders because of a fall in total peripheral resistance. In nonresponders, total peripheral resistance did not fall. It is suggested that nonresponders developed irreversible structural or functional vascular changes responsible for maintained resistance elevation. Urinary sodium excretion fell markedly and sodium balance became less positive in all pigs. On low sodium, plasma renin activity and aldosterone remained depressed in DOCA-treated pigs, but rose steadily in control pigs. Serum sodium was unchanged in all pigs, but the hypokalemia seen in DOCA-treated pigs on normal sodium was reversed with low sodium. High water turnover seen in all DOCA-treated pigs was always reversed by the low-sodium diet. It is concluded that in some animals on a low sodium diet, BP is lowered to normotensive levels, whereas in others it is not. The failure of a low sodium diet to produce a fall in arterial pressure in these animals is not related to changes in the renin-angiotensin system, in serum potassium levels, or water turnover rates. (Hypertension 2: 490-496, 1980)

KEY WORDS • DOCA hypertension • sodium restriction • renin-angiotensin system • hypokalemia • water turnover

It is well known that the development of mineralocorticoid hypertension is largely sodium-dependent. Although young rats1 and pigs2 on a standard sodium diet become hypertensive following large doses of deoxycorticosterone acetate (DOCA), most animal models require supplemental sodium in either their food or drinking water to achieve substantial blood pressure (BP) elevation.1,3-5

The ability of sodium restriction to reverse the hypertensive process is less well established. Although it is generally assumed that lowering dietary sodium will reduce BP in most hypertensive subjects,6 reversal of the hypertensive process does not always occur.4,7 Furthermore, in long-standing DOCA hypertension, the BP does not fall after cessation of DOCA and high sodium administration. This condition of persistent pressure elevation has been called "metacorticoid hypertension" and has been reviewed.8

We have studied the effects of dietary sodium deprivation on DOCA hypertensive pigs to determine if a low sodium diet always reverses high BP to normotensive levels in this model, and to monitor hemodynamic, metabolic, and endocrine variables during sodium depletion so as to characterize associated factors.

Methods

Animal Care and Instrumentation

Twelve young male feeder pigs (Yorkshire White) were used for this study. The animals were housed in 4 × 4 ft metabolic cages and given food and water ad libitum. Each pig ate approximately 1.5 kg Purina Pig Chow (112 mEq Na+ and 191 mEq K+/kg) per day, and no salt was added to the food or drinking water.

Each animal was subjected to two major operations. Thoracic surgery was performed to place an electromagnetic flowprobe (Zepeda Instrument Company) around the ascending aorta for cardiac output.
measurements and to implant a Tygon catheter in the aortic arch just distal to the flowprobe for arterial pressure recording. Following recovery from this surgery, a laparotomy was performed to implant a Tygon catheter in the inferior vena cava with its tip in the central venous pool. Catheters and flowprobe leads were exteriorized on the left side near the eighth rib and tied to a wire that was looped under the skin. This instrumentation was protected by a heavy canvas jacket. Operative details are presented elsewhere.

Hemodynamic Measurements

Aortic and venous pressures and cardiac outputs were monitored daily in each pig. Because of difficulties in terminal flowprobe calibrations, cardiac outputs are expressed as percent change in millimeter of pen deflection instead of absolute flow values. Total peripheral resistance was calculated as the quotient of mean arterial pressure (MAP) divided by cardiac output.

Metabolic Measurements

Water intake, urine output, and food intake were measured daily. Dietary sodium and potassium (mEq/24 hr) values were calculated and urinary electrolyte concentrations were measured on a National Instrument Laboratories flame photometer with an internal standard of lithium. Water and electrolyte balances (dietary intake-urinary output) were calculated.

Blood Sampling

Blood for endocrine determinations was collected on ice. The plasma was separated by centrifugation and frozen until assayed. Plasma renin activity (PRA) was measured by radioimmunoassay of angiotensin I generated during a 30-minute incubation. Serum aldosterone was also determined by radioimmunoassay using antisera directed against aldosterone-17-lactone. Blood for determination of sodium and potassium concentrations was collected in plastic tubes and centrifuged immediately at room temperature. Plasma was refrigerated until assayed.

DOCA Implantation

Following a stabilization period post surgery, nine pigs received subcutaneous implantations of DOCA (Sigma Chemical), 100 mg/kg body wt, impregnated in a Silastic (Dow Corning) matrix, 1:2 by weight. Three control pigs received Silastic implants without DOCA. These implantations were made on the left flank with the pig under light thiamylal sodium (Surital, Parke-Davis) anesthesia.

Low Sodium Intervention

Following DOCA implantation, MAP steadily rose in all pigs. This pressure rise was complete by 2-3 weeks. At that time, the animals were placed on a “normal sodium” powdered diet for 4-12 days. The concentration of sodium in this diet was 121 mEq/kg; that of potassium was 253 mEq/kg. This change in diet was given to prepare the animals for the altered consistency of a powdered “low sodium” diet (10.1 mEq Na/kg). The “low sodium” diet was usually given for 22 days. However, since one animal received the diet for only 17 days and because major changes in metabolic, endocrine, and hemodynamic variables were complete within this period of time, the data are presented for just the first 17 days. The time from implant to the beginning of sodium restriction ranged from 15 to 21 days for Silastic control pigs and from 29 to 54 days for DOCA-treated pigs.

Analysis of Data

The data are expressed as means ± SEM. Comparisons of hemodynamic, metabolic, and endocrine variables were made between control and DOCA hypertensive pigs on given days. Variables were also compared for each group before the low sodium intervention (Days —4 to —1) and afterward (Days +11 to +17). Statistical significance of difference between and within groups was determined by Student's t test.

Results

Effects of DOCA and Silastic implantation on hemodynamic, metabolic, and endocrine variables in our pigs are presented in table 1. The MAP rose in all DOCA-treated pigs, initiated by increases in cardiac output alone, total peripheral resistance alone, or a mixture of the two. Central venous pressures were slightly elevated in all DOCA-treated pigs, and heart rates were unchanged. Control pigs showed no changes in arterial BP, central venous pressure, or heart rate following implantation.

Following DOCA implantation, serum DOC rose 20-fold within the first 24 hours and remained elevated for at least 2 months thereafter. By Day 10 following implant, plasma renin had fallen to levels that could not be measured. Serum aldosterone (not shown) was suppressed, but to a lesser degree. Water turnover increased dramatically in these animals, with no significant change in water balance. Serum sodium remained constant following implantation; however, serum potassium fell. Since hypokalemia occurred at a time when potassium balance remained unchanged (there was no kaluresis in these animals), it was inferred that there was a shift of potassium intracellularly. No important changes in these variables occurred in control pigs.

From these steady-state levels, a lowered sodium diet produced the hemodynamic, endocrine, and metabolic changes in DOCA hypertensive and control pigs described below.

Hemodynamics

Based on the changes in arterial pressure resulting from the low sodium diet, two clearly different groups of pigs could be distinguished. In five pigs, BP fell to within 5% of normotensive levels (responders); in four others (nonresponders), BP remained at least 30%
higher than normal (fig. 1). Pre-low sodium mean arterial pressures of the responders fell from 134 ± 2 to 101 ± 2 mm Hg on Days 11-17 of low sodium (135% ± 1% to 103% ± 1% of pre-DOCA implant pressure). Nonresponder pressures fell from 145 ± 8 to 136 ± 3 mm Hg (from 150% ± 2% to 140% ± 2% of pre-DOCA implant pressure). Pre-low sodium pressures were significantly higher in nonresponders than in responders (p < 0.01). Aortic pressure of control pigs did not change from preimplant values at any time during the postimplant or low sodium periods.

Neither responders nor nonresponders showed a change in cardiac output while on the low sodium diet (table 2). Since cardiac output was the same in all DOCA hypertensive pigs and not different from pre-low sodium values, BP fell in responders because of a fall in total peripheral resistance. In nonresponders, the total peripheral resistance did not fall (table 2). The time from DOCA implant to the onset of low sodium intervention ranged from 27 to 54 days (mean = 38 days) in the responders and from 29 to 48 days (mean = 39 days) in the nonresponders.

Central venous pressures in the DOCA-treated pigs did not fall significantly when the animals were placed on a low sodium diet. There was no difference in the degree of change between responders and nonresponders. Central venous pressure in the control animals did not change significantly throughout the low sodium study. Heart rates of all animals on a low sodium diet were unchanged from pre-low sodium values.

### Endocrines

Plasma renin activities of control pigs climbed steadily from 2.6 ± 0.6 to 12.9 ± 2.0 ng AI/ml/hr, whereas plasma renin activities of all DOCA-treated pigs remained depressed for the duration of the low sodium intervention. Plasma renin activities of DOCA-treated responders, however, became measurable by Days 11-17 whereas those of the nonresponders did not (table 3). Plasma aldosterone levels paralleled those of plasma renin for each group of animals (table 3).

### Electrolytes and Water

Urinary sodium excretion (fig. 2) fell precipitously and equivalently in all three groups of pigs within the first 24 hours on the low sodium diet, confirming the equivalent diminution in sodium intake in the three groups. Sodium balances, which were highly positive in these growing pigs on the standard sodium diet,
FIGURE 1. Mean arterial pressure changes in DOCA-hypertensive and control pigs on a low sodium diet. Data are expressed as % of pre-implant mean arterial pressure. Circles represent mean values obtained on a single day and bars are one standard error of the mean. Closed circles are control pigs; open circles + solid lines are responder pigs; and open circles + dashed lines are nonresponder pigs.

FIGURE 2. Urinary sodium excretion in DOCA-hypertensive and control pigs on a low sodium diet. Data are expressed as mEq urinary sodium/24 hr. Circles represent mean values obtained on a single day. Closed circles are control pigs; open circles + solid lines are responder pigs; and open circles + dashed lines are nonresponder pigs.

TABLE 3. Endocrine Changes in DOCA-Hypertensive and Control Pigs on a Low Sodium Diet

<table>
<thead>
<tr>
<th>Pig type</th>
<th>Plasma renin activity (ng AI/ml/hr)</th>
<th>Aldosterone (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-low sodium Days 11-17</td>
<td>Pre-low sodium Days 11-17</td>
</tr>
<tr>
<td>Control (n = 3)</td>
<td>2.6 ± 0.6</td>
<td>6.5 ± 0.5</td>
</tr>
<tr>
<td>Responders (n = 5)</td>
<td>&lt;0.02</td>
<td>4.4 ± 1.1</td>
</tr>
<tr>
<td>Nonresponders (n = 4)</td>
<td>&lt;0.02</td>
<td>3.4 ± 1.0</td>
</tr>
</tbody>
</table>

*Significantly greater than control values, pre-low sodium (p < 0.001).
†Significantly greater than non-responder values, days 11-17 (p < 0.01).
became less positive in all three groups so that little, if any, sodium was retained. Serum sodium levels did not change measurably throughout the study in any of the three groups (table 4).

Serum potassium in control pigs remained unchanged throughout the study. All DOCA-treated pigs were hypokalemic on the standard sodium diet (table 1). The hypokalemia of both responders and nonresponders returned toward normal values within the first few days on the low sodium diet and remained normal for the duration of the study (table 4). Potassium balances were equally positive in all three groups of pigs and not measurably changed by the low sodium diet.

The water turnover, which was very high in all DOCA-treated pigs before they were placed on the low sodium diet (table 1), was reversed by this procedure in both responders and nonresponders (fig. 3). Water intake of DOCA-treated responders fell from 19.1 ± 0.8 liter/24 hr to 5.7 ± 0.6 liter/24 hr on Days 11–17. Nonresponder intakes fell from 21.0 ± 0.5 to 5.2 ± 0.4 liter/24 hr. Water turnover values of the control pigs were unchanged during the low sodium diet (3.4 ± 0.2 to 3.3 ± 0.1 liter/24 hr). Urine volumes paralleled water intake so that water balance was unchanged and not different among any of the three groups.

Discussion
Effects of Low Sodium Treatment in Hypertension

Although patients with mild hypertension have been successfully treated with low dietary sodium,5, 12, 13 the effects of dietary sodium restriction on DOCA-salt hypertension have not been clearly defined. It has been shown that "metacorticoid" hypertension — a self-sustaining arterial pressure elevation that is no longer dependent upon a continuing administration of DOCA — is resistant to sodium restriction.5 Musilova et al.14 studied rats given 1% NaCl to drink and made hypertensive by injections of microcrystalline suspensions of DOCA. When the salt water was replaced with tap water 10 weeks following the termination of DOCA administration, the hypertension was reversed. De Champlain et al.15 have observed that rats made hypertensive with weekly subcutaneous injections of DOCA and 1% NaCl in their drinking water exhibit a BP fall following the cessation of DOCA and salt administration. Furthermore, when other DOCA hypertensive rats were placed on a sodium deficient diet, their hypertensions were reversed despite continued DOCA administration. Our present study is the first to characterize hemodynamic, electrolyte, and endocrine changes that occur in DOCA-dependent hypertension in response to low sodium treatment.

Hemodynamic Changes

Results of this study show that some DOCA hypertensive animals maintain an elevated arterial BP in the presence of reduced dietary sodium, whereas others do not. The observation that hypertension is reversible in some subjects but not in others on a low sodium diet is not a novel one. Dole et al.16 reported that treatment of "benign and uncomplicated" hypertension by lowering dietary sodium from 180 to 7 mEq/24 hr resulted in the return to normotensive BP levels in just 60% of their patients. Experimentally, Swales and Tange17 reported evidence for two types of renal hypertensive rats. They showed that one-kidney Goldblatt hypertensive rats responded to acute sodium depletion by becoming normotensive, whereas two-kidney Goldblatt hypertensive rats did not.

Since nonresponders did not decrease total peripheral resistance on the low sodium diet (table 2), it is possible that these animals have developed irreversible changes in the walls of their resistance vessels. Support for this possibility comes from the fact that the pre-low sodium mean arterial pressures of nonresponders were significantly higher than those of responders (fig. 1). Such a situation would be compatible with adaptive structural changes in the vascular wall due to an increased pressure load.18 It has been observed that vascular changes exist in pigs with hypertension resulting from DOCA treatment identical to that of the current study. Such changes include increases in vascular smooth muscle sensitivity and increases in structural resistance.19 The maintained vascular resistance in the nonresponders of this study may have been due to a persistence of either or both of these changes. Since the time from implant to low sodium treatment was not different between responders and nonresponders, it is possible that certain animals are intrinsically more predisposed to these "vascular changes." Three of four animals that

<table>
<thead>
<tr>
<th>Pig type</th>
<th>Pre-low Na⁺ (mEq/l)</th>
<th>Pre-low K⁺ (mEq/l)</th>
<th>Days 11–17</th>
<th>Days 11–17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>142 ± 1.0</td>
<td>5.1 ± 0.9</td>
<td>5.6 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>(n = 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responder</td>
<td>147 ± 2.4</td>
<td>3.7 ± 0.2</td>
<td>5.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonresponder</td>
<td>144 ± 2.2</td>
<td>3.3 ± 0.3</td>
<td>4.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>(n = 4)</td>
<td></td>
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</tr>
</tbody>
</table>

*Significantly greater than pre-low sodium values (p < 0.05).
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FIGURE 3. Water intake in DOCA-hypertensive and control pigs on a low sodium diet. Data are expressed as liters of water drunk in 24 hr. Circles represent the mean values obtained on a single day and bars are 1 standard error of the mean. Closed circles are control pigs; open circles + solid lines are responder pigs; and open circles + dashed lines are nonresponder pigs.

subsequently became responders (one of the five responders had a nonfunctional flow probe) developed their hypertension by an increase in cardiac output, whereas all four pigs that were to become nonresponders had initial BP elevations that were dependent upon increases in total peripheral resistance.

Endocrine Changes

Whereas control pigs showed a steady rise in plasma renin activity while on low sodium, plasma renin levels remained markedly depressed in all DOCA-treated pigs throughout most of the postimplant and all of the low sodium period. This observation argues against a role for the renin-angiotensin-aldosterone system in the maintenance or regression of hypertension in this model.

Water and Electrolyte Changes

The high water turnover in mineralocorticoid hypertension18,19 was reversed in all DOCA-treated pigs in this study. Since nonresponders showed an equivalent fall in water turnover with no change in BP, it is evident that the polydipsia-polyuria that accompanies DOCA hypertension in this model is merely coincident with and not necessary for the maintenance of sustained BP elevation. DOCA hypertension can develop in the absence of increased water turnover.20,21 However, the persistence of hypertension after the termination of an elevated turnover has not been previously reported.

The hypokalemia observed in all pigs was reversed in DOCA-treated pigs on a low sodium diet (table 4). Since it has been suggested that the original hypokalemia was the result of a shift of potassium into cells,11 it appears that this intracellular movement of potassium was at least partially sodium-dependent. Additionally, since hypokalemia was reversed in all DOCA pigs, the maintenance of high BP seen in some pigs was not dependent upon low levels of potassium in the blood.

Serum sodium levels were not significantly changed throughout the study (table 4). Since there were no differences between values obtained from responders and nonresponders, it seems likely that major changes in serum sodium are not necessary for the regression of hypertension seen in this study.

Urinary sodium excretion fell and sodium balance became less positive when the pigs were subjected to the low sodium diet. The fact that sodium excretion was decreased in these animals was not surprising and has been reported previously.3,4 However, the time course of the fall was quite abrupt (fig. 3). In each group of animals, sodium excretion fell dramatically within the first 24 hours of dietary sodium restriction. This happened without an apparent change in serum sodium. An obvious explanation for changes seen in control pigs is that an increase in activity of the renin-angiotensin-aldosterone system contributes to the fall of urinary sodium.22 However, this cannot explain the phenomenon seen in DOCA-treated animals, where the renin-angiotensin system has been rendered inoperative and changes in aldosterone concentration would have been masked by high concentrations of DOCA.

The changes observed in the measured variables of this study permit some insight into the mechanisms that may be responsible for the reversal of the pressure elevation with lowered dietary sodium. We considered that either or both of the following processes may play a role in this phenomenon: 1) The reduction in the positive sodium balance in our growing pigs, which occurs in the absence of measurable changes in serum sodium or in water balance, is compatible with the possibility that intracellular sodium concentration had fallen. Such transmembrane distribution changes of
sodium have been implicated in the etiology of hypertension and may play a role in the hemodynamic changes observed in our study. 2) Alternatively, since water turnover rates were so high just prior to low sodium treatment, a slight, but important decline in water balance during low sodium may have gone unnoticed. If water balance fell along with sodium balance, this would argue that extracellular fluid volume had fallen. This volume change might also be responsible for the observed changes in a number of variables that occur during low sodium.

It is concluded that some animals are unable to achieve a lowered BP while on a low sodium diet. The failure of a low sodium diet to produce a fall in arterial pressure in these animals is not related to changes in the renin-angiotensin system, in serum potassium levels, or in water turnover rate.

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