Electrolyte and Hormonal Effects of
Deoxycorticosterone Acetate in Young Pigs

ROGER J. GREKIN, M.D., JAMES M. TERRIS, M.D., AND DAVID F. BOHR, M.D.

SUMMARY Balances of sodium, potassium, and water were studied in the growing male pig as hypertension developed in response to subcutaneous implantation of deoxycorticosterone acetate (DOCA). Serum sodium, potassium, deoxycorticosterone (DOC), aldosterone, and plasma renin activity (PRA) were determined. These variables were observed in a total of 10 experimental and nine control pigs. All animals were uninephrectomized and fed a diet of Purina Pig Chow and tap water ad libitum. No salt was added to the food or water. Serum DOC levels rose dramatically on the day of the implantation, then gradually declined but remained approximately 10 times greater than control levels 40 days after implant.

Plasma renin activity was suppressed rapidly and completely, whereas aldosterone fell only slowly to about half its control value. Sodium retention was maximum during the first 24 hours. Thereafter an "escape" process became operative, causing sodium balance to return to normal after the third day, at which time the major rise in arterial pressure occurred. A marked increase in water turnover (intake and output) also began after the third day and persisted throughout the experimental period. Water balance remained normal during this period of increased turnover. Hypokalemia developed in the absence of kaliuresis, suggesting that potassium moved into the cells. Except for the potassium retention, these changes parallel the abnormalities seen in other states of mineralocorticoid excess. (Hypertension 2: 326-332, 1980)

KEY WORDS • Deoxycorticosterone acetate (DOCA) • pig • sodium • hypokalemia • mineralocorticoid hypertension • hormones • electrolytes • water balance • renin

EXCESSIVE mineralocorticoid and salt produce experimental hypertension in several species. This hypertension is commonly characterized by transient sodium and water retention, potassium wasting, hypokalemia, and alkalosis. Despite the large number of studies performed upon these experimental models, the pathogenesis of hypertension remains controversial.

We have developed an animal model for mineralocorticoid hypertension using deoxycorticosterone acetate (DOCA) implantation in pigs, which offers several advantages for the study of hypertension. These include the rapid and highly reproducible elevation in blood pressure and the relatively large size of the animal, which allows for accurate hemodynamic measurements. In this report we describe the electrolyte and hormonal changes seen during the development of DOCA hypertension in pigs.

Methods and Materials

Animals

Young male feeder pigs (Chester White or Yorkshire, unneutered), 20 to 30 kg, were purchased from local farmers. All animals were chronically instrumented for a number of studies as previously described. After 5 to 10 days of stable baseline measurements, the animals were anesthetized with thiamylal (Surital), and underwent subcutaneous insertion (in the left flank) of strips of DOCA in silicone rubber (experimental n = 10) or silicone rubber alone (control n = 9). DOCA implants were prepared by mixing DOCA (Sigma Chemical Co.) with liquid silicone rubber (Dow Corning Silicone) in a ratio of 1:2 by weight. The total dose of DOCA was 100 mg/kg body weight.

Endocrine Determinations

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. Antiserum was obtained from Diagnostic Products Corporation, Culver City, California. Plasma was extracted with dichloromethane, and the
extract was placed on a silica gel column. The column was eluted with dichloromethane twice, then with dichloromethane:methanol (100:9). The final eluate was dried and used in the assay system. Recovery averaged 70%. Cross reactivity for DOC was less than 0.001%.

Plasma DOC was measured by radioimmunoassay using DOC antiserum obtained from Dr. Guy Abraham, Torrance, California. After adding \(^{3}H\)DOC for recovery, samples were extracted twice with ethyl ether and separated by freezing the aqueous layer in a dry ice-acetone mixture. The ether extract was decanted, dried, and reconstituted in isooctane saturated with ethylene glycol, and applied to a celite column (0.5 g celite mixed with 0.25 ml ethylene glycol). The columns were washed with isooctane and eluted with isooctane:ethyl acetate (85:15). The eluate was dried, reconstituted in ethanol, and applied on Whatman No. 1 paper. The paper was coated with 25% propylene glycol in acetone, and samples were chromatographed in toluene saturated with propylene glycol. The DOC area was identified by radiochromatogram scanner and extracted with ethanol. These extracts were then assayed by radioimmunoassay. Recovery averaged 50% with a nondetectable blank and intra-assay variation of less than 5%.

Metabolic Determinations

For metabolic studies the pigs were housed in specially constructed 4 × 4 ft metabolic pens and given a daily ration of Purina Pig Chow and water ad libitum. No salt was added to the food or water. The daily sodium intake from the Purina diet was approximately 150 mEq. Salt and water balances were computed on the basis of 24-hour urine collections and measured food and water intake. In addition to the 24-hour collections, intake and output were measured in 8-hour segments in some experimental and some control animals from 2 days preimplant to 6 days postimplant. Complete balance studies including fecal sodium and potassium determinations were performed in one DOCA and one control pig.

Electrolyte Determinations

Concentrations of sodium and potassium in urine and blood were measured on a National Instrument Laboratories flame photometer with an internal standard of lithium (14.41 mEq lithium/liter). Blood samples were collected in plastic tubes on ice. Serum was separated by centrifugation and samples were frozen until assayed. Fecal electrolytes were determined by dehydrating and ashing the stool, followed by taking up the residue in 1N HNO₃.

Statistical Measurements

Comparison of sodium, potassium, and water intake, output and balance was made between DOCA and control pigs before and following implantation. Since measurements were made in the same experimental animals repeatedly over time, overall protection levels for each variable were determined using the Bonferroni inequality test and the Student \(t\) test.

Results

All DOCA pigs had an increase in mean arterial pressure within 3 days of implantation (fig. 1). Pressure continued to rise progressively during the first 10 days after implant, then became stable at values approximately 40% greater than control. Details of pressure and hemodynamic changes associated with the development of hypertension are described in a separate report.⁹

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Figure 1. Mean arterial pressure in experimental and control animals. DOCA-treated animals had an elevation in arterial pressure within 48 hours of implantation. Pressure remained elevated throughout the course of the study. Values are expressed as mean ± SEM.
Endocrine Measurements

By 4 hours following implantation, serum DOC had risen from a pre-implant level of 112 ± 37 ng/dl to 1718 ± 256 ng/dl. By 8 hours, levels peaked at 2482 ± 240 ng/dl, and fell to 1326 ± 330 ng/dl by Day 2. The level continued to fall gradually, reaching values between 520 and 680 ng/dl by Day 40. DOC levels in the control pigs ranged from 10 to 140 ng/dl (fig. 2 left).

Plasma renin activity in DOCA-treated pigs reached a level significantly lower than controls by day 3 after implant (fig. 2 center). By day 20, the PRA was less than 0.1 ng/ml/hr in all samples and did not increase throughout the remainder of the study period. Serum aldosterone in DOCA-treated pigs did not differ from the controls during the first six days following implantation (fig. 2 right). Thereafter, moderate suppression was seen with levels of aldosterone in DOCA-treated pigs being less than 50% of control.

Electrolytes and Water

Values for intake, output, and balances of sodium, potassium, and water are illustrated in figures 3, 4, and 5. Figure 6 depicts urinary sodium, potassium, and water excretion rates determined on an 8-hour basis for the 2 days prior to and 6 days following implantation. Fecal electrolytes were not measured in most of the animals, and we have used the term "balance" to describe the difference between intake and urinary output of sodium and potassium. All

Figure 2. Endocrine changes during the development of DOCA hypertension. Serum DOC levels rose dramatically immediately following implantation of DOCA. Despite a subsequent gradual fall, serum DOC levels remained markedly elevated in experimental animals. Plasma renin activity was suppressed in DOCA-treated pigs beginning 3 days after implantation. Suppression was sustained throughout the experimental course. Serum aldosterone was suppressed to about 50% of control levels starting 10 days post-implantation of DOCA. All values are expressed as mean ± SEM.

Figure 3. Sodium metabolism in DOCA and control pigs. DOCA-treated pigs had a 3-day period of decreased urinary sodium output immediately following implantation. This decrease in output was reflected in an increased positive sodium balance. Thereafter, sodium output and balance returned to control levels.
Figure 4. Potassium metabolism in DOCA and control pigs. There were no significant differences in potassium balance between experimental and control groups. Mild potassium retention was seen during the first 3 days following implantation in experimental animals, kaliuresis was not observed.

Figure 5. Water metabolism in DOCA and control pigs. DOCA treated animals had a decrease in urine volume with water retention during the first 3 days following implantation, although the values were not significantly different than those for control animals. Water intake and urine volume rose steadily in experimental pigs following the fourth day after implantation.
values are expressed on a per-kilogram-body-weight basis. Because the pigs were growing throughout the study, all balances tended to be positive. For the two pigs evaluated with fecal electrolyte determinations, fecal sodium represented 10–15% of total sodium output, and remained constant throughout the experimental period. Fecal potassium also remained constant at 15–25% of total output.

Sodium intake and urinary sodium output were stable throughout the 8-day period prior to DOCA implantation (fig. 3). On the day of implantation there was a dramatic decrease in sodium output, which was evident during the first 8 hours (fig. 6). Sodium excretion in DOCA-treated pigs during the first 2 days after implant was significantly less than that seen for control pigs \((p < 0.02)\). Output began to rise after 24 hours, with a gradual return to preimplant values, which was complete by Day 3. Sodium intake remained relatively constant throughout. During the first 2 days following implantation, DOCA-treated pigs had a total positive sodium balance of \(6.08 \pm 0.57 \text{ mEq/kg} \) as compared to \(2.71 \pm 1.12 \text{ mEq/kg} \) in control pigs \((p < 0.025)\), giving an excess sodium retention of \(3.37 \text{ mEq/kg} \) in the treated pigs. From Day 3 to 40, both control and DOCA-treated pigs remained in positive sodium balance with no significant differences between the two groups. Since serum sodium remained constant throughout the study, excessive sodium retention appears to have been accompanied by retention of isotonic volumes of water. The average weight of experimental pigs at implant was 33.9 kg. The calculated excess sodium retention of \(3.37 \text{ mEq/kg} \) indicates that each pig retained an average of 114.2 mEq sodium during the initial 2 days of sodium retention. This would represent an increase of 761 ml of isotonic extracellular fluid (ECF) volume. Assuming an initial ECF volume of 20% of total body weight, or 6.8 liters, 761 ml represents an increase of 11% in ECF volume.

Changes in potassium handling were parallel to the changes in sodium (figs. 4 and 6). There was a decrease in urinary potassium in DOCA-treated pigs during the first 2 days following implantation. There was no significant difference in potassium intake in the two groups. During the first 2 days following implantation, DOCA pigs had a positive potassium balance of \(6.96 \pm 0.88 \text{ mEq/kg} \). Positive potassium balance in control pigs for the same period was \(3.65 \pm 1.36 \text{ mEq/kg} \) \((p < 0.05)\). Following Day 3, intake, output, and balance of potassium in DOCA-treated pigs were not different than controls.

Serum potassium concentration in DOCA-treated pigs was lower than in controls prior to implant, and steadily decreased throughout the first 6 days following implantation. Following the first week, potassium remained between 2.5 and 3.0 mEq/l compared to values of 4.8 to 5.8 mEq/l in control pigs. This depression of serum potassium occurred at times when the potassium balance appeared more positive in DOCA-treated than control animals. Serum sodium was the same in both groups, and no significant changes were seen during the course of the study.

Water intake and urine volume were stable for 1 week before DOCA implantation (fig. 5). Paralleling changes in sodium and potassium, urine volume was decreased for the first 3 days following implantation (fig. 6). DOCA-treated pigs had a positive water balance of \(129.6 \pm 9.7 \text{ ml/kg} \) for the first 2 days after implant, while control pigs had a positive balance of \(105.2 \pm 16.5 \text{ ml/kg} \). Although this was not a significant difference, the excess water retention in DOCA-treated pigs for the first 2 days was 828.5 ml per animal, quite close to the figure calculated on the basis of sodium retention.

After the first 4 days, urine volume steadily rose over a 15-day period to values three to four times greater than control values \((p < 0.001)\) (fig. 5). Water intake remained constant in DOCA-treated pigs during the first 4 days following implantation, then steadily rose in a fashion parallel to urine output. Intake was significantly greater than in controls from Day 8 to 35 \((p < 0.001)\). From Day 3 to 40, water balance was variable, but similar to preimplant balance and to that of control pigs.
Weight Measurements

Weight gain in the DOCA-treated pigs during the preimplant period was less than that in the controls, though not significantly so. During the first few days after implant, it equalled or exceeded that in the controls, and thereafter it was once again less. Whereas the preimplantation weight gain of the DOCA-treated pigs was 13.8 g/kg/day, that for the first 2 days after DOCA implantation was 23.6 g/kg/day. This greater weight gain of 9.8 g/kg/day or 760 g/pig in 2 days can be reasonably attributed to the increased water retention, which has been estimated as 761 ml or 828 ml by independent measurements of sodium and water balance respectively.

Discussion

Subcutaneous implantation of DOCA impregnated in silicone in young pigs has proven to be a reliable and convenient means of producing hypertension. Serum levels of DOC were consistently elevated within the first few hours after this implantation and remained elevated 40 days or more. The suppression of renin following DOCA implantation occurred after a 2-day period of sodium retention, and was similar to the suppressed PRA seen in other mineralocorticoid excess states. Despite the rapid fall in renin, plasma aldosterone levels were not different from controls during the first week after implant, and subsequent suppression of aldosterone in DOCA pigs was never as complete as the concomitant suppression of renin. This was unexpected, as DOCA administration in man results in prompt suppression of aldosterone. Systematic studies of the control of aldosterone release have not been done in pigs, but in other species potassium and ACTH are both known secretagogues of aldosterone. Potassium levels fell following implantation and cannot be implicated in the continued secretion of aldosterone following renin suppression. ACTH levels were not measured, but the considerable stress associated with handling may well have resulted in ACTH-mediated secretion of aldosterone.

The pattern of early sodium retention followed by escape is similar to that seen in other forms of mineralocorticoid hypertension. Sodium retention precedes and may be necessary for the development of hypertension. It should be noted, however, that the major pressure elevation occurs between Days 3 and 14 at a time when sodium balance does not differ between experimental and control animals. Whereas many animal models require administration of supplemental sodium, this was not necessary to produce hypertension in pigs. The daily sodium intake was high, however, in the range of 4.5 mEq/kg/day. The speed and degree with which sodium retention and hypertension occur are striking, in all likelihood secondary to the very high serum levels of DOC that are achieved. DOC is estimated to be 10% as effective as aldosterone as a sodium-retaining steroid and probably has very little importance under most physiologic conditions. The levels achieved in this study, however, are probably high enough to cause near maximal mineralocorticoid receptor occupancy, leading to marked and rapid sodium retention.

The consistent decrease in urinary potassium excretion following implantation is surprising. Mineralocorticoids have potent kaliuretic effects in rat, dog, and man, and renal potassium loss has been used as a marker of mineralocorticoid activity in bioassay systems. However, noted decreased urinary potassium and positive potassium balance in rabbits that were administered aldosterone. Despite positive potassium balance, rabbits rapidly become hypokalemic, suggesting an intracellular shift of potassium. A similar mechanism may account for the hypokalemia seen in pigs in the absence of kaliuresis, although increased fecal potassium losses cannot be excluded. In the one DOCA-treated pig for which we have fecal potassium determinations, increases in fecal potassium did not occur.

It is not entirely unexpected that the effects of mineralocorticoid upon potassium differ between species while sodium retention is a constant feature; mineralocorticoid actions on sodium and potassium handling by the kidney appear to be mediated by different cellular mechanisms. Fimognari et al. have shown that sodium retention is inhibited by actinomycin D and appears to depend upon steroid-induced new protein synthesis. Kaliuresis is not blocked by actinomycin D, and may be effected by intracellular events unrelated to new protein synthesis.

Increased water turnover was described in some of the earliest reports of experimental DOCA hypertension, but the mechanism remains unclear. Potassium depletion inhibits urinary-concentrating mechanisms, and may lead to nephrogenic diabetes insipidus. The severity of the defect in concentrating ability is usually fairly mild, and would not be expected to lead to the massive polyuria seen in these experiments. Berl et al. have demonstrated an effect of potassium depletion upon the thirst center, leading to primary polydipsia.

This model provides an experimental tool to study the mechanisms of the hypokalemia that occurs despite potassium retention, the marked increase in water turnover, the escape from mineralocorticoid-induced sodium retention, and the suppression of renin.

Rapid development of hypertension in the DOCA-treated pig appears to follow sodium retention associated with an increase of approximately 11% in extracellular fluid volume. Since the sodium retention is complete within 3 days following DOCA implantation and the major rise in arterial pressure occurs following this 3-day period, an immediate causal role for sodium retention in the genesis of hypertension must be questioned.

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