Red Blood Cell Sodium in the DOCA Hypertensive Pig

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SUMMARY The influence of deoxycorticosterone acetate (DOCA) on the sodium content of the red blood cell was determined in the pig. DOCA (100 mg/kg), impregnated in Silastic, was implanted subcutaneously (s.c.) in six male pigs; seven additional pigs received Silastic implants without the DOCA. Those receiving DOCA had an increase in mean arterial pressure (MAP) that was significant in 48 hours and reached a plateau that was 24 mm Hg greater than that of the controls after 15 days. These animals also developed hypokalemia and polydipsia over approximately the same time course. Red blood cell sodium content increased in DOCA-treated pigs 24 hours after implant (5.57 ± 0.17 vs 5.23 ± 0.05, mEq/liter cells). The sodium content continued to rise, reaching a plateau 28% above that of control value by the 5th post implant day (6.37 ± 0.40 mEq/liter cells). In vitro tests of possible mechanisms that might have caused the in vivo increase in red blood cell sodium content gave the following results: 1) Incubations of red blood cells in a physiological salt solution (PSS) containing deoxycorticosterone failed to cause an increase in cell sodium content. 2) No ouabain-like factor was demonstrated in plasma from the DOCA hypertensive pigs. 3) An elevation in bicarbonate concentration in the PSS caused an increase in red blood cell sodium content. 4) A decrease in potassium concentration in the PSS also caused an increase in red blood cell sodium content. We conclude that the increase in red blood cell sodium content that occurs in the DOCA hypertensive pig could be due, at least in part, to either the increase in plasma bicarbonate or to the hypokalemia that occurs when this animal develops DOCA hypertension. If a similar change in cell sodium content occurs in hypothalamic centers that regulate the cardiovascular system and thirst, this could play a role in DOCA-induced hypertension and polydipsia. (Hypertension 5 (supp V): V-105-V-109, 1983)

KEY WORDS • hypokalemia • ouabain-like factor • polydipsia • sodium pump

SINCE in hypertension the level of arterial pressure often varies with the amount of sodium ingested, this element is thought to play an important role in some types of experimental and clinical hypertension. The mechanism by which sodium causes this effect is not known, but it is the central theme of much research.1,2

Investigators studying altered electrolyte metabolism of the red blood cell in hypertension have considered the possibility that this abnormality reflects changes that may occur in other tissues such as vascular smooth muscle or the central nervous system, in which the abnormality may play a causal role in the elevated arterial pressure.3,4

In the current study, we have observed that the development of deoxycorticosterone acetate (DOCA) hypertension in the pig is accompanied by an increase in intracellular sodium content in the red blood cell. Additional studies were carried out to explore possible mechanisms by which DOCA may produce this increase.

Materials and Methods

DOCA hypertension was produced in three young, male, feeder pigs obtained from a local supplier and in three adult male Yucatan miniature pigs obtained from Buckshire Corporation, Perkasie, Pennsylvania. Four feeder pigs and three miniature pigs served as controls. Arteries and veins were catheterized to measure blood pressure and to draw blood samples. DOCA (100 mg/kg, Sigma Chemical Company, St. Louis, Missouri) impregnated in strips of Silastic was implanted subcutaneously to produce hypertension. No sodium was added to the drinking water but the animals received approximately 200 mEq sodium per day in their Purina Pig Chow. Silastic without DOCA was implanted in control pigs.

For red blood cell sodium and potassium determinations, 10 ml blood samples were drawn into heparinized syringes from arterial catheters. Blood was centrifuged (immediately) at 1700 × g for 3 minutes. Plasma and buffy coat were aspirated, and plasma was saved for sodium and potassium determinations by flame photometry. Then 12 to 15 ml of an ice-cold solution of 110 mM MgCl₂ and 10 mM Tris-HCl (pH 7.4 at 25°C), with an osmolarity adjusted to 295 mOsm, was used to wash 1.5 ml of packed red blood cells. This wash was repeated once.

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Aliquots of packed cells (0.5 ml) were suspended in 1 ml of the MgCl₂ solution. Duplicate microhematocrit determinations were made of this suspension. Then 0.5 ml of this suspension was pipetted into 4.5 ml (1 in 10 dilution) of a hypotonic lysing solution containing 16.7 mEq/liter LiNO₃. The sodium concentration in this solution of lysed cells was determined by flame photometry. Cell sodium content was calculated by multiplying the determined sodium concentration by the dilution factor and dividing by the fraction of packed red blood cells. For each blood sample, determinations were done on the hemolysates from three 0.5 ml aliquots of packed cells, and the results averaged.

In vitro incubation studies were performed to determine the effects of deoxycorticosterone, potassium, or bicarbonate on the sodium content of the red blood cells from normotensive pigs. Washed packed red blood cells (1 ml) was added to 14 ml of physiological salt solution (PSS) in a 25 ml siliconized glass Erlenmeyer flask, which was sealed with parafilm. Flasks were placed in a Lab-line shaker bath and gently shaken to prevent sedimentation. Incubations were carried out at 37°C for 1 to 4 hours. The PSS had the following composition (in mM): NaCl (140), KCl (5.0), MgSO₄ (1.2), Na₂HPO₄ (1.2), MOPS (2), CaCl₂ (1.6), dextrose (11.1), and bovine serum albumin (0.25%, Sigma). The pH was adjusted to 7.4 at room temperature with NaOH. Sodium concentrations of the packed red blood cells and potassium concentrations of the PSS were determined on aliquots of the suspension taken before and after the incubation.

The direct in vitro effect of deoxycorticosterone (DOC, Sigma) was studied by adding this steroid to the PSS to give concentrations ranging from 10⁻⁸ to 10⁻⁴ M. Ethanol was used to dissolve the DOC and was present in both test and control samples in a 0.1% concentration.

The in vitro effect of hypokalemia was studied by incubating the red blood cells in PSS containing potassium in concentrations ranging from 0.1 to 5.0 mM. The time course of the hypokalemia effect was studied by observing the cell sodium content at 1, 2, and 4 hours.

For studies of the effect of bicarbonate concentration on the red blood cell sodium content, the cells were incubated in PSS containing 0.26, and 52 mM bicarbonate. The pH was kept constant by titrating the PSS with O₂ containing 0, 5, or 10% CO₂, respectively. The time course of the bicarbonate effect was studied by determining the red blood cell sodium content at 1, 2, and 4 hours.

The presence of an ouabain-like factor in the plasma of the DOCA-hypertensive and Silastic control pig was bioassayed utilizing the rabbit red blood cell net sodium and potassium fluxes. The plasma from volume-expanded (20% isotonic expansion of extracellular volume) normotensive pigs was also bioassayed for the ouabain-like factor. This insured reliability of the bioassay, since this factor has been previously reported in volume-expanded animals. Blood (50 ml) was taken on ice from control and experimental pigs and prepared by a modification of the method of Gruber and Buckalew. Briefly, the blood was centrifuged for 20 minutes at 2500 × g and the plasma removed. The plasma was allowed to remain at room temperature for 30 minutes then boiled for 5 minutes. The boiled fraction was then centrifuged for 60 minutes at 10,000 × g, and the supernatant was placed in Amicon filters (25,000 dalton molecular weight exclusion limit) and centrifuged for 45 minutes at 750 × g, and the ultrafiltrated boiled plasma was collected. Sodium, potassium, and osmolality of the ultrafiltrate was adjusted to equal the PSS used in the measurement of rabbit red blood cell fluxes (potassium = 3.0 mM; sodium = 140 mM; osmolality = 295 mOsm). Rabbit red blood cells, obtained by cardiac puncture, from ketamine-anesthetized male New Zealand white rabbits were incubated in these ultrafilters or in PSS with or without ouabain. Sodium pump activity during a 3-hour incubation period at 37°C was estimated by measuring red blood cell sodium content in aliquots of the suspension fluid at the beginning and end of this incubation.

Statistical evaluation of the data was by the Student's t test for unpaired samples. The 0.5 level of probability was regarded as significant.

Results

Pigs receiving DOCA implants developed hypertension, hypokalemia, and an increase in red blood cell sodium content following a time course illustrated in figure 1. Although we did not observe a significant change in plasma sodium concentration in this series of pigs, we did observe a slight but significant increase beginning about 10 days after DOCA implantation in a previous study. In addition, these pigs all developed the polydipsia that we have previously reported. This began approximately 4 days after DOCA implantation and by the end of 1 month, the water intake was approximately four times normal.

Preimplant value for red blood cell sodium content for the pigs that were to receive DOCA was 5.1 ± 0.3 mEq/liter packed cells; this value for control pigs was 5.1 ± 0.2 mEq/liter. Red blood cell sodium content increased in DOCA-treated pigs 24 hours after implant (5.57 ± 0.17 vs 5.23 ± 0.05 mEq/liter cells). Sodium content in these cells continued to increase for 5 days, stabilizing at approximately 1.2 mEq/liter above control values for the remainder of a 30-day observation period. No significant change in red blood cell sodium content occurred in control pigs. In one miniature pig, the subcutaneous DOCA was removed 30 days after implantation. Red blood cell sodium content fell to preimplant value within 2 days. Other relevant variables also returned rapidly to control values.

One mature miniature pig was placed on a low salt diet (10 mEq/day) at the time of DOCA implantation. In this animal following DOCA implantation, no change occurred in red blood cell sodium content or in the other relevant variables. However, 20 days after the DOCA implantation, sodium in the diet was restored (200 mEq/day), and this restoration was fol-
Factors that Increase Red Blood Cell Sodium Content

A. Low external potassium concentrations

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>[K⁺] (mM in PSS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td>221</td>
<td>0</td>
</tr>
<tr>
<td>223</td>
<td>0.4</td>
</tr>
<tr>
<td>avg inc</td>
<td>0.4</td>
</tr>
</tbody>
</table>

B. Elevated external bicarbonate concentrations

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>[HCO₃⁻] (mM in PSS; pH constant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>220</td>
<td>0.1</td>
</tr>
<tr>
<td>231</td>
<td>0.1</td>
</tr>
<tr>
<td>avg inc</td>
<td>0.1</td>
</tr>
</tbody>
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Cells were incubated for 2 (bicarbonate) or 3 (potassium) hours at 37°C in the appropriate PSS. Values are expressed as absolute increase from preincubation values in mEq Na/liter cells.

TABLE 2. Factors that Increase Red Blood Cell Sodium Content In Vitro

Data are means of triplicate determinations, expressed in mEq/liter packed cells. Incubation was for 4 hours at 37°C.

Several of the parameters that changed following DOCA administration were examined in vitro for their effects on red blood cell sodium content. First, the effect of the mineralocorticoid itself was evaluated. Deoxycorticosterone base (DOC) was used instead of DOCA because of its greater water solubility. In Table 1, data are presented from in vitro studies in which red blood cells from normotensive pigs were incubated in PSS or PSS with DOC (10⁻⁸, 3 × 10⁻⁷, or 10⁻⁵ M) for 4 hours at 37°C. The red blood cells incubated in DOC-PSS had a sodium content that was not different from those incubated in PSS alone.

A second variable to be elevated in vitro for its possible effect on red blood cell sodium content was the extracellular potassium concentration. It is evident in figure 1 that the development of hypokalemia parallels the increase in red blood cell sodium content. In the in vitro studies, red blood cells from normotensive pigs were incubated for 3 hours at 37°C in PSS containing potassium in concentrations ranging from 0.1 to 5.0 mEq/liter. Table 2 A data demonstrate that the red blood cell sodium content varied inversely with the external potassium concentration. Over the range of potassium concentrations occurring in the pig in response to DOCA administration, a reduction of potassium concentration of 1 mEq/liter resulted in an increase in sodium content ranging from 0.2 to 0.4 mEq/liter packed cells. However, time course studies (not shown) demonstrated that 3-hour incubation was insufficient for the cell to reach a new steady level of sodium content.

The results of using 0, 26, and 52 mM bicarbonate in the red blood cell incubations are shown in Table 2 B. The pH of all samples was kept constant at 7.4 ± 0.05 by using 0, 5, and 10% CO₂, respectively, in the O₂ used for aeration. Following a 2-hour incubation, the red blood cell sodium content increased 0.1, 0.4, and 1.0 mEq/liter cells respectively, with the increasing bicarbonate concentrations. However, again the red blood cell sodium content had not reached a steady state value even after 4 hours when incubated in 26 and 52 mM bicarbonate PSS.

A fourth variable that may influence intracellular sodium content is a ouabain-like factor that has been reported to be present in the plasma of "volume expansion" forms of hypertension. This factor might act as does hypokalemia by decreasing sodium pump activity. To test for the presence of such a factor, rabbit red blood cells were incubated in plasma ultrafiltrate for 3 hours at 37°C. Figure 2 presents data that compare the effects on red blood cell pump activity of plasma ultrafiltrate from normotensive control pigs, DOCA hypertensive pigs, and volume-expanded normotensive pigs. Also presented are data comparing the effects on pump activity of incubation of these red blood cells in control PSS with that of incubation in PSS containing 10⁻³ M ouabain. Plasma from DOCA hypertensive pigs did not inhibit pump activity, where-
as that from the volume-expanded normotensive pigs produced 44% ± 4% of the inhibition produced by the maximal concentration of ouabain.

Discussion

The results of these studies demonstrate that the intracellular sodium content of red blood cells rises significantly during the development of mineralocorticoid-induced hypertension in the pig. It remains elevated during the maintenance phase of this hypertension. This finding is in agreement with Zumkley \(^{14}\) who reported an increase in red blood cell sodium content in DOCA-salt hypertensive rats. However, the correlation of the time course of the increase in blood pressure and the increase in red blood cell sodium content was not previously demonstrated. Both the elevation in sodium content of the red blood cell and the elevation in arterial pressure are evident 48 hours after DOCA implantation and continue to rise throughout the first week. When the DOCA implant is removed, both variables begin to decline within 48 hours. Neither occurs with DOCA administration when the pig is on a low salt diet. These chronologic parallelisms between blood pressure elevation and the increase in red blood cell sodium content constitutes a basis for considering that the two changes may be based on a common etiological mechanism.

The following mechanisms were investigated as possible causes of the rise in red blood cell sodium content: 1) direct action of deoxycorticosterone; 2) sodium pump inhibition by a plasma-borne factor; 3) hypokalemia; and 4) an increase in plasma bicarbonate concentration. The first two of these possible mechanisms were demonstrated to have little influence on the sodium content of red blood cells as studied in vitro and therefore of little probable significance in vivo. Although mineralocorticoids have been known to induce an increase in membrane sodium channels, \(^{15}\) this requires protein synthesis with mineralocorticoid action on the nucleus. \(^{16}\) The lack of a direct action of mineralocorticoids on red blood cell sodium content has also been demonstrated by Stern et al. \(^{17}\) in cells from normotensive subjects.

The second mechanism that we investigated as a cause for the rise in red blood cell sodium content is the presence of a plasma-borne ouabain-like factor. Extensive work done in this area has shown that such a factor is present in volume-expanded forms of hypertension. \(^{8,13}\) Songo-Mize et al. \(^{18}\) have recently presented evidence that such a factor is present in the plasma of DOCA-salt hypertensive rats. In our studies, we found clear evidence for the presence of a sodium-pump-inhibiting factor present in normotensive pigs that have been acutely volume expanded by 20% of their extracellular fluid volume. However, in preliminary studies on plasma filtrate from three of our DOCA-hypertensive pigs, we failed to demonstrate the presence of a factor that depressed sodium pump activity (fig. 2). Further observations are needed to evaluate the possible role of this factor in the DOCA-hypertensive pig.

The presence of both hypokalemia and increased plasma bicarbonate concentration in vivo correlate well with results on the changes in red blood cell sodium content produced by these parameters in vitro. Levin et al. \(^{19}\) have reported an increase in red blood cell sodium content in patients with hypokalemia. Asstrup \(^{20}\) has observed increases in human red blood cell sodium content when plasma potassium levels fell below 3.5 mEq/liter. Glynn \(^{21}\) had established that these results are due to partial pump inhibition in the red blood cell. The influence of bicarbonate concentration on red blood cell sodium content has been investigated by Funder and Wieth. \(^{22}\) They reported an increase in sodium content in red blood cells from patients with metabolic alkalosis. They also observed this increase in sodium content in red blood cells from normal subjects incubated in high bicarbonate solutions. Our present in vitro studies have corroborated these findings using the pig red blood cell. We have demonstrated that in this species the cell sodium content is also increased by either a decrease in potassium or by an increase in bicarbonate concentration in the PSS. These electrolyte changes have been observed in the plasma of the DOCA-hypertensive pig. In vivo we have observed that plasma potassium fell approximate-
ly 1.2 mEq/liter, and plasma bicarbonate rose approximately 8 mEq/liter (unpublished observation) following DOCA treatment. The data from our in vitro studies do not permit a quantitative assessment of the effect of shifts of these magnitudes on the steady state content of sodium in the red blood cell. However, it is probable that either a decrease in potassium or an increase in bicarbonate concentration, or both, could be responsible for the observed increase in red blood cell sodium content in vivo following DOCA implantation in the pig.

Although the observed increase in red blood cell sodium content probably plays no role in the pathogenesis of hypertension, the mechanism responsible for this increase may be important. We have hypothesized that an increase in intracellular sodium content may occur in hypothalamic arterial pressure regulating centers and that this increase may reset the center to stabilize arterial pressure at a higher level. We now hypothesize that the same mechanisms that are responsible for the increase in red blood cell sodium content in mineralocorticoid hypertension are responsible for the electrolyte change in the hypothalamic pressure regulating center that causes the hypertension.

References

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