Altered Ion Transport in Aortic Smooth Muscle during Deoxycorticosterone Acetate Hypertension in the Rat

By Allan W. Jones and Robert G. Hart

ABSTRACT

Changes in aortic water and electrolyte distribution and in ion turnover were studied during the development of deoxycorticosterone acetate (DOCA) hypertension in the rat. Treatment with DOCA plus saline during the prehypertensive phase was associated with increases in "K turnover (0.0142 ± 0.0005 vs. 0.0102 ± 0.0003 min⁻¹), cell water (0.89 ± 0.03 vs. 0.76 ± 0.02 kg/kg dry weight), and the ratio of weight to length. These parameters were further increased during the development of hypertension. Significant increases were also observed in total K, Ca, and Mg contents and in Na and Cl contents corrected for the extracellular space. The turnover of 36Cl was increased (0.230 ± 0.006 vs. 0.136 ± 0.004 min⁻¹) in DOCA hypertensive rats as was the content of slowly exchanging Cl. Removal of extracellular Ca greatly increased the steady-state turnover of "K. For control rats, a Ca concentration of 0.1 DIM reduced the rate of 42K turnover to less than 50% of the Ca-free value (0.063 ± 0.004 min⁻¹), whereas DOCA hypertensive rats exhibited only a 10% reduction. At the highest Ca concentration, 5 IDM, the "K turnover was greater in DOCA-treated rats with the hypertensives operating at 67% of maximum efflux or about twice the efflux in controls. It is concluded that significant alterations in ion transport by vascular smooth muscle occur before and during the development of hypertension induced by treatment with DOCA plus saline. Such changes may result from a reduced ability of Ca to stabilize the membrane. It is proposed that such alterations contribute to the changes in vascular reactivity and the hypertrophy associated with hypertension.

• The events associated with the development of hypertension of various forms include abnormalities in arterial geometry and in water and electrolyte contents (1-3). Because comparisons have generally been made between controls and groups with established hypertension, little opportunity has been available to evaluate cause and effect relations. Recently, however, Hansen and Bohr (4) have found similar increases in the reactivity of femoral arteries taken from high- and low-pressure legs in deoxycorticosterone acetate (DOCA) hypertensive rats; this finding supports the concept that changes in excitability can occur independently of increases in wall tension resulting from high blood pressure. It has been noted that altered reactivity may result from an increase in or a resetting of membrane transport mechanisms (5). Using radioisotope techniques, increases in sodium (Na), potassium (K), and chloride (Cl) turnover have been found in various arteries from spontaneously hypertensive rats (5-7). The application of an ion-exchange approach using lithium (Li) substitution for external Na at 2°C has indicated that the replacement of cell Na and K by Li is accelerated in tail arteries from DOCA hypertensive rats (8). Little evidence is available, however, to establish whether such alterations in ion transport precede pressure changes or occur after the establishment of hypertension.

Abnormalities in vascular calcium (Ca) metabolism have also been implicated in the pathogenesis of hypertension. The ability of high external Ca concentrations to depress K-induced contractures is reduced in DOCA, renal, and spontaneously hypertensive rats (4, 9). Hinke (10) has reported that perfused tail arteries of DOCA hypertensive rats maintain greater K-induced contractures in low Ca concentrations (0.2-0.4 M) than do tail arteries of control rats. Acutely, Ca removal causes a greater increase in 42K efflux in the aortas of spontaneously hypertensive rats than in controls (7). These observations indicate that a common pattern of increased ion permeability or "leakiness" in the vascular smooth muscle, perhaps resulting from a decrease in the ability of Ca to control permeability at normal levels (7, 9), is associated with at least some forms of hypertension (5-8). Further evidence is needed, however, to establish an altered dependence of steady-state ion turnover on external Ca concentration during hypertension.

The primary objectives of the present study were...
(1) to investigate the time course of aortic changes during the induction of hypertension with DOCA plus saline treatment and (2) to evaluate the effects of altered external Ca concentration on the steady-state turnover of K. It was anticipated that the application of steady-state radioisotope techniques coupled with tissue analyses of water, electrolytes, and extracellular spaces on adjacent segments would yield sufficient resolution to separate extra-cellular and smooth muscle changes.

Methods

Animal and Tissue Preparation.—Male Wistar rats weighing 250–300 g routinely underwent unilateral nephrectomy and were placed on one of several regimes. Control rats for the initial experiments were given tap water ad libitum, and controls for later experiments were placed on 1.0% (w/v) saline. The steroid-treated groups were injected intramuscularly twice a week with 6 mg of DOCA suspended in sesame oil. The DOCA hypertensive rats were given saline or, in one series of experiments, saline supplemented with 0.2% (w/v) KCl. All of the rats were given normal rat chow. Rats were routinely weighed during the treatments, and fluid intake was periodically determined by 24-hour removal from calibrated drinking bottles. The DOCA-treated rats received their last injection 3–4 days before experimentation. Rats were killed by a blow to the head and bled, and the entire thoracic aorta was placed in a K- and Ca-free dissection solution at 37°C. Loose connective tissue was removed, and the aorta was cut along its length. A segment along the intercostal region was measured to the nearest millimeter, mounted on a stainless steel holder, and placed in an isotope solution for equilibration. The upper aorta and the aortic arch were used for the determination of water and electrolyte content and distribution after equilibration in the physiological solution for 3 hours (11).

Solutions.—The solutions used were similar to those employed previously (6). The normal physiological solution had the following millimolar composition: Na* 146.2, K* 10.0, Mg** 1.2, Ca** 2.5, CI* 148.9, HCO3- 13.6, H2PO4- 1.2, and glucose 5.7. Solutions were buffered with 97% O2-3% CO2 at 37°C resulting in a pH of 7.4. K and Ca were omitted for dissection, which reversibly depleted tissue K. The physiological solution for determining extracellular space contained 46CoEDTA (0.2–0.5 μCi/ml) plus 60CoEDTA (2 μM) (11).

Isotope Techniques.—These procedures have been employed previously (5–7). Briefly, aortas were equilibrated with the isotope for 3–5 hours. After a 1–2-second rinse, the strips were passed through a series of tubes containing nonradioactive solution. The activity in the tubes and the aorta was counted in a gamma well counter (42K) or by liquid scintillation techniques (46Cl). Tissue 46Cl was released by ashing in H2O2 (30% w/v); the ash was then dissolved in physiological solution to minimize quenching differences between tissues and washout tubes. Washout curves were calculated by sequentially adding the tissue and tube counts in reverse order and normalized in terms of percent initial activity (11).

The extracellular space was determined from the uptake of 46CoEDTA after 15 minutes of incubation (11). The 46CoEDTA space was calculated on the basis of the ratio of counts/sec in the strip to counts/sec in a weighed sample of the experimental solution. The space was represented in terms of kg H2O/kg dry weight. "Cell" water was taken as the difference between total water and 46CoEDTA space. Similarly, the cell electrolyte content was estimated as the difference between the total electrolyte content and that dissolved in the 46CoEDTA space. The amount of K and Cl characterized by slow turnover was derived from the isotope (counts/sec kg-1) remaining in the tissue after a 1-minute washout and the specific activity (μmoles/count sec-1) of the isotope solution.

Electrolyte Analysis.—Tissues were lightly blotted and placed in plastic tubes for weighing. Weight-length ratios were calculated by dividing wet weight (mg) by length (cm). Water contents were determined by weight differences after oven drying (20 hours at 93°C). Tissues undergoing analysis for Ca and magnesium (Mg) were ashed at 85°C in H2O2 (30% w/v) containing AgNO3 to precipitate the Cl-. The ash was dissolved in a solution containing 0.1n HNO3, 10 mM La3+ and 15 mM Li in NO3 form. Mg, Ca, and Ag were analyzed on an atomic absorption spectrometer (Instrumentation Laboratory, IL 453) and Na and K on a flame photometer (IL 153). Cl was calculated from the difference between total Ag added and final Ag in the extract (11). Contents are presented in terms of μmoles/kg wet weight or dry weight.

Blood Pressure.—Mean arterial blood pressure was determined through a catheter in the tail artery under light ether anesthesia 1–3 days before experimentation. A Statham P23AC transducer and a Brush 220 rectilinear recorder were employed.

Data Processing.—A digital computer was used to process the data for the isotope washout experiment (11). Steady-state turnover, k, was calculated as described previously (6) from the time, t, required for the slow component to progress to 1/e of the initial percent (determined by extrapolation to zero time or from the counts remaining after 1 minute). Turnover, k = 1/t, represents the fraction of the isotope washed out per minute and has units of min-1. This approach yields an accurate description of a component following a single homogeneous exponential or one exhibiting a statistically distributed turnover (12). In one series of experiments, the efflux of K was calculated from the product of the turnover (min-1) and the size of the electrolyte pool exhibiting that turnover (μmoles/kg wet weight). Under the steady-state conditions of these experiments, in which the size of the electrolyte pool remained constant (net flux equaled zero), efflux was taken to equal influx (13). Significance was determined by Student’s t-test.

Results

Effects of Various Regimes.—The short-term effects (2 weeks) of the fluid and DOCA treatments are shown in Figure 1. Over this period, blood pressure was unaffected by the four combinations. Fluid intake was significantly elevated in rats receiving saline alone (P < 0.001). DOCA treatment was associated with an additional increase in
Effects of fluid and DOCA treatments of 2 weeks duration on rat blood pressure, fluid intake, and \(^{40}\)K turnover in aortas. Small bars indicate ± SE with the number of rats observed appearing in parentheses. S = saline.

saline consumption \((P < 0.005)\). The turnover of aortic K was significantly elevated only in the group receiving DOCA plus saline \((P < 0.001)\). These results indicate that an important interaction occurs between DOCA and saline treatment, resulting in significant changes in vascular K turnover.

Timed Study of DOCA Hypertension.—Continued treatment with DOCA plus saline led to a significant elevation in blood pressure over a 2-6-week period \((P < 0.01)\); maximum blood pressures were recorded by 8 weeks (Fig. 2). There was a progressive rise in saline consumption over the first 6-week period, but a plateau was reached between 6 and 8 weeks. In contrast to the time course of the changes in blood pressure, relatively large changes occurred in aortic smooth muscle during the prehypertensive period. The turnover of K was elevated by 40% at 2 weeks \((P < 0.001)\). Cell water was expanded by approximately 17% \((P < 0.01\), Fig. 2 and Table 1\) and the weight-length ratio for the aorta was increased by a similar percent at 2 weeks. During the period of increasing blood pressure (2-8 weeks), there was a marked hypertrophy of the aorta, as indicated by a 50% increase in the weight-length ratio, in comparison to controls of similar age. Cell water also increased during this period but to a lesser extent, with hypertensives exhibiting an expansion of 32%. The change in \(^{40}\)K turnover proceeded more slowly after 2 weeks (Fig. 2 and Table 1).

The time courses of the treatment effects on aortic water and electrolytes determined under in vitro conditions are shown in Table 1. Unlike cell water, which increased, the \(^{60}\)CoEDTA space underwent little change. Significant increases occurred in total K, Mg, and Ca contents during treatment. Although changes in total Na and Cl were not significant, when corrections were made for the extracellular space, a consistent increase was seen. Cell K concentration (total K – \(^{60}\)CoEDTA space K/cell H\(_2\)O) was not significantly altered throughout the treatment.

The rats on the DOCA plus saline treatment did not achieve the same weight gain as did the control rats on saline (Fig. 3). This lower weight gain may have influenced the small drop in blood pressure seen between 8-12 weeks (Fig. 2). K depletion occurs under conditions of Na loading and high mineralocorticoid levels \((14)\). Several of the treated rats exhibited anorexia, dyspnea, and general skel-
TABLE 1

Water and Electrolyte Contents and Distribution in Incubated Rat Aortas during DOCA Plus Saline Treatment

| Time  | &*CoEDTA Total H₂O (kg/kg dry wt) | H₂O space (kg/kg dry wt) | Cell H₂O (kg/kg dry wt) | Total Na (mmoles/kg dry wt) | Total K (mmoles/kg dry wt) | Total Mg (mmoles/kg dry wt) | Total Ca (mmoles/kg dry wt) | Total Cl (mmoles/kg dry wt) | Na (mmoles/kg dry wt) | K (mmoles/kg dry wt) | Cl (mmoles/kg dry wt) | Cell K (mmoles/kg H₂O) | Cell K turnover (min⁻¹) |
|-------|---------------------------------|---------------------------|-------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------|-------------------|-------------------|---------------------|----------------------|----------------------|
| Control | 8  | 2.64 ± 0.06 | 1.89 ± 0.06 | 0.76 ± 0.02 | 324 ± 11 | 134 ± 2 | 10.6 ± 0.3 | 16.9 ± 0.3 | 346 ± 12 | 48 ± 5 | 114 ± 2 | 64 ± 5 | 151 ± 3 | 0.0102 ± 0.0003 |
| 2 weeks   | 7  | 2.64 ± 0.08 | 1.75 ± 0.09 | 0.89 ± 0.03 | 314 ± 14 | 132 ± 4* | 11.9 ± 0.6 | 17.5 ± 0.7 | 350 ± 19 | 64 ± 3* | 134 ± 4* | 88 ± 11 | 151 ± 7 | 0.0142 ± 0.0006* |
| 8 weeks   | 9  | 2.73 ± 0.04 | 1.72 ± 0.05 | 1.01 ± 0.04* | 343 ± 11 | 170 ± 4* | 13.4 ± 0.4* | 18.8 ± 0.5* | 343 ± 12 | 85 ± 4* | 153 ± 4* | 80 ± 6 | 147 ± 4 | 0.0156 ± 0.0008* |
| 12 weeks  | 10 | 2.89 ± 0.06 | 1.87 ± 0.05 | 1.02 ± 0.03* | 356 ± 7 | 167 ± 6* | 13.1 ± 0.5* | 19.8 ± 0.5* | 384 ± 14 | 83 ± 4* | 148 ± 6* | 99 ± 6 | 145 ± 4 | 0.0161 ± 0.0006* |

All values are means ± se.
* P < 0.001.
† P < 0.01.

To test the possible effects of K depletion and associated acid-base complications on the previous observations (Table 1 and Fig. 2), a series of rats was treated with DOCA plus saline supplemented with 0.2% (w/v) KCl. These rats maintained weight gains equivalent to those of control rats (Fig. 3). Their mean blood pressure of 141 ± 5 mm Hg (N = 8) at 6 weeks was similar to that of DOCA plus saline-treated rats as was their aortic weight-length ratio of 16.0 ± 0.5 mg/cm (N = 8). The turnover of 42 K from the KCl-supplemented DOCA series was greater than that of controls receiving saline alone (Fig. 4). The turnover of 42 K from the KCl-supplemented DOCA series during the same period (Table 3, &*CoEDTA 42 K turnover) was greater than that for saline-supplemented rats studied during the same period (Table 2, &*CoEDTA 42 K turnover). Chloride turnover was measured in a negative control series (Fig. 5). The isotope removed after 1 minute has been previously identified as being primarily of smooth muscle origin (6). As shown on Table 2, marked alterations in membrane trans-
ION TRANSPORT AND DOCA HYPERTENSION

Steady-state washouts of $^{42}$K from aortas of saline-treated control rats (circles, $n = 8$) and DOCA + saline + KCl-treated hypertensive rats (squares, $n = 8$). Counts are expressed as a percent of initial counts (log scale) and plotted vs. time. Lines indicate a single exponential with a turnover of $0.0098 \pm 0.0002$ min$^{-1}$ in controls vs. $0.0219 \pm 0.0021$ min$^{-1}$ in DOCA hypertensives. The points are averages $\pm$ SE except where the size of the symbol exceeds the SE.

Control of K Turnover by Ca.—It is well known that Ca plays an important role in modulating membrane properties such as ion permeability. A series of experiments was conducted to test the hypothesis that the increased turnover of K is related to a reduced ability of extracellular Ca to stabilize the membranes of vascular smooth muscle from DOCA plus saline-treated rats. The tissues were equilibrated with $^{42}$K in solutions containing the same Ca concentration as that in the nonradioactive washout solution. The effects of various extracellular Ca concentrations on steady-state $^{42}$K turnover are shown in Table 3. The turnover was greatly accelerated in Ca-free solution with controls exceeding DOCA plus saline-treated rats. Increasing the extracellular Ca concentration to 0.1 mM reduced turnover in controls to less than 50% of the Ca-free value, whereas the hypertensives exhibited only a 10% reduction. The 50% response for the DOCA plus saline-treated group (for an asymptote of 0.020 min$^{-1}$) was estimated to be at an extracellular Ca concentration of 0.6 mM. Over the higher Ca range, the $^{42}$K turnover for the hypertensive rats exceeded that for the control rats.

Changes in extracellular Ca concentration also induced significant alterations in aortic electrolyte contents. At low extracellular Ca concentrations, Na was elevated and K was reduced compared with their levels at normal Ca concentrations. Treatment with DOCA and saline augmented this effect, as indicated by the greater decrease in K content when the extracellular Ca concentration was reduced from 5.0 to 0.1 mM. In general, as K turnover increased with reduced extracellular Ca concentration, the ability of smooth muscle to maintain K content was reduced. This effect might result from an upper limit for the number of moles of K that can be transported inward to replace the K moving out of the cell under the steady-state conditions of this experiment. In this light, K efflux was calculated (slow $^{42}$K content times turnover, Table 3); it appears that a maximum of about 1.0–1.2 mmoles/kg wet weight min$^{-1}$ was reached. At a normal extracellular Ca concentration, the efflux for control rats was 40% of maximum. However, the
TABLE 2
Chloride Turnover in Aortas from Rats Treated with Saline or DOCA Plus Saline for 8 Weeks

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Turnover (min⁻¹)</th>
<th>Slowly exchanging ³¹Cl (mmoles/kg dry wt)</th>
<th>(mmoles/kg cell H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>6</td>
<td>0.136 ± 0.004</td>
<td>38.4 ± 1.3</td>
<td>48.0 ± 1.6</td>
</tr>
<tr>
<td>DOCA + saline</td>
<td>6</td>
<td>0.230 ± 0.006*</td>
<td>56.3 ± 2.0*</td>
<td>55.7 ± 2.0†</td>
</tr>
</tbody>
</table>

All values are means ± se.
*P < 0.001.
†P < 0.025.

hypertensive rats were operating at or in excess of 67% of maximum efflux.

Discussion

Control of normal excitability of vascular smooth muscle and reactivity to hormones and transmitters requires the maintenance of stable membrane permeability characteristics. The induction of hypertension with DOCA and saline is associated with increases in the turnover of cell K and Cl. Such parallel changes are consistent with depolarization of the membrane (5, 15). These changes are also in the same direction as those induced by norepinephrine (5, 6), which depolarizes vascular smooth muscle, probably via a general increase in membrane permeability (5, 16). The increase in ⁴²K turnover during DOCA hypertension is similar to that induced by approximately 5 x 10⁻¹⁰ g/ml of norepinephrine in control aortas (6). Thus, the altered membrane transport properties noted in the present study represent a significant perturbation of the normal vascular control system in the direction of increased peripheral resistance. This finding is consistent with the observation of Bohr and co-workers (4, 9) that dose-contracture responses are shifted to lower concentrations of epinephrine and KCl for arteries from DOCA hypertensives. Shifts associated with DOCA hypertension are larger than those associated with spontaneous hypertension (4, 9). Likewise, the changes in ⁴²K and ³¹Cl turnover for DOCA-treated rats exceed those in spontaneously hypertensive rats (6).

Other changes are associated with DOCA hypertension which could influence altered reactivity. The increased concentration of Cl in cell H₂O is consistent with a partial depolarization of the membrane (15). Elevations in Na (corrected for ⁶⁰CoEDTA space) and total tissue Ca are consistent with the findings of others for DOCA treatment (10, 17). Although some of this material may be bound to mucopolysaccharides (18, 19), such sites offer only limited capacity (6, 11). Significant alterations in smooth muscle behavior would result if part of the ionic shift (in excess of binding) were intracellular. Increased cell Na has been associated with increased contractile responses related to shifts in Na-Ca transport (20, 21). Since intracellular Ca concentration is one of the principle factors limiting contractile responses in smooth muscle (22), any ionic shift that increases cell Ca, either directly or indirectly, can contribute to increased

TABLE 3
Effects of Extracellular Calcium Concentration, [Ca]₀, on Electrolyte Content and Potassium Turnover in Rat Aorta

<table>
<thead>
<tr>
<th>[Ca]₀ (mM)</th>
<th>Treatment</th>
<th>N</th>
<th>Total Na (mmoles/kg wet wt)</th>
<th>Total K (mmoles/kg wet wt)</th>
<th>Slow ⁴²K turnover (min⁻¹)</th>
<th>⁴²K efflux (mmoles/kg wet wt min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Saline</td>
<td>7</td>
<td>103.4 ± 1.9</td>
<td>20.8 ± 1.4</td>
<td>0.063 ± 0.004</td>
<td>1.00 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>DOCA + saline</td>
<td>7</td>
<td>113.0 ± 1.3*</td>
<td>19.2 ± 1.4</td>
<td>0.050 ± 0.003*</td>
<td>0.75 ± 0.13</td>
</tr>
<tr>
<td>0.1</td>
<td>Saline</td>
<td>6</td>
<td>90.1 ± 1.2</td>
<td>34.6 ± 0.7</td>
<td>0.030 ± 0.003</td>
<td>0.92 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>DOCA + saline</td>
<td>8</td>
<td>96.4 ± 2.6</td>
<td>33.0 ± 2.9</td>
<td>0.045 ± 0.003*</td>
<td>1.22 ± 0.14</td>
</tr>
<tr>
<td>1.0</td>
<td>Saline</td>
<td>6</td>
<td>83.2 ± 3.2</td>
<td>38.5 ± 2.0</td>
<td>0.0123 ± 0.0005</td>
<td>0.40 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>DOCA + saline</td>
<td>8</td>
<td>93.8 ± 4.9</td>
<td>38.4 ± 3.8</td>
<td>0.0275 ± 0.0026†</td>
<td>0.90 ± 0.19†</td>
</tr>
<tr>
<td>2.5</td>
<td>Saline</td>
<td>7</td>
<td>79.8 ± 2.5</td>
<td>38.3 ± 0.8</td>
<td>0.0099 ± 0.0003</td>
<td>0.34 ± 0.01†</td>
</tr>
<tr>
<td></td>
<td>DOCA + saline</td>
<td>8</td>
<td>88.0 ± 1.9*</td>
<td>42.1 ± 1.4*</td>
<td>0.0219 ± 0.0021†</td>
<td>0.81 ± 0.06†</td>
</tr>
<tr>
<td>5.0</td>
<td>Saline</td>
<td>7</td>
<td>82.4 ± 3.0</td>
<td>37.6 ± 0.8</td>
<td>0.0105 ± 0.0006</td>
<td>0.36 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>DOCA + saline</td>
<td>7</td>
<td>83.3 ± 2.7</td>
<td>45.5 ± 1.4†</td>
<td>0.0195 ± 0.0018†</td>
<td>0.82 ± 0.07†</td>
</tr>
</tbody>
</table>

All values are means ± se.
*P < 0.05.
†P < 0.01.
‡ Saline supplemented with 0.2% (w/v) KCl.
reactivity. More information concerning the distribution of Na and Ca during DOCA treatment is needed.

The time study allowed separation of some prehypertensive events from hypertensive changes. Altered K turnover preceded the development of elevated blood pressure as did relatively small changes in cell H2O and the weight-length ratio. Saline consumption was also elevated before the onset of hypertension. The volume expansion associated with Na loading has been proposed to play an important role in the pathogenesis of hypertension via the resulting increases in blood volume and cardiac output (23, 24). It is difficult to envision how such changes could lead to altered ion turnover under in vitro conditions in which differences between oxygen, wall tension, catecholamines, and angiotensin II levels are minimized. Another possible sequence of events during DOCA and saline treatment could focus on the arterial wall in which altered permeability would give rise to increased reactivity to normal levels of vasoactive agents. Progressive hypertrophy of the vascular wall at "controller" sites (e.g., the aortic arch) would lead to a resetting of the cardiovascular controls so that normal levels of autonomic activity would persist in the face of increasing blood pressure. Such resetting has been associated with established renal and spontaneous hypertension (25, 26).

The vascular changes appeared to be independent of severe alterations imposed on the rat's K balance during treatment with DOCA plus saline. Supplemental KCl countered the weight loss and the associated symptomatology, confirming earlier work (27), but the progress of the vascular changes and the hypertension at 6 weeks was if anything augmented. Providing supplemental KCl in studies that involve prolonged treatment with DOCA would ensure a more stable animal.

In the present study, saline provided an important adjunct to DOCA in producing vascular changes. The necessity of saline for the maximum blood pressure effect is well known (28), but the means by which saline interacts with DOCA is at present unclear. Perhaps the changes in body fluid homeostasis induced by the large consumption of saline affect other hormonal systems capable of vascular effects. Specific systems have yet to be identified, however.

The membrane stabilization action of Ca is generally accepted (22, 29, 30). It was, therefore, not surprising that the turnover of vascular K was closely dependent on the concentration of extracellular Ca. K turnover was greatly elevated in Ca-free solutions as noted previously (7, 31). It is of interest to note that the steady-state turnover of K in Ca-free conditions was slightly higher for controls than it was for DOCA hypertensives, indicating that the unstabilized membrane properties were similar. The Ca concentration of 0.1 mm required to reduce turnover in controls to 50% is similar to that required to effect a 50% change in normal canine carotid artery selectivity to K over Na (31). The requirement for higher Ca concentrations to reduce K turnover during DOCA treatment supports the hypothesis that the increased ion turnover in the hypertensives is related to a decreased ability of Ca to stabilize the membrane of vascular smooth muscle. For the Ca concentration range employed, it is not clear whether very high Ca levels reduce K turnover to normal levels or whether the two curves approach different asymptotes. Studies involving extended ranges of divalent ion concentrations and membrane stabilizing agents may provide the information needed to deduce whether the abnormality is related to a shift in the affinity or conformation of membrane sites for binding Ca or to a deficiency of sites for control.

The decreased ability of Ca to stabilize ion turnover is consistent with the observations of a reduced ability of high Ca concentrations to inhibit KCl contractures in femoral arteries of the rat (4, 9). Hansen and Bohr (4) have also noted that the decreased ability of Ca to inhibit contractures is similar in arteries exposed to high blood pressure and those protected by iliac ligation. Their conclusion that elevated blood pressure is not an essential signal for reactivity changes is confirmed in the present study by the finding of increased K turnover during the prehypertensive period.

Another profound alteration in ion transport was seen in the Ca study. The steady-state efflux of cell K in DOCA hypertensives for an extracellular Ca concentration of 2.5 mm was about 67% of maximum or double the control level. The cell K concentration was only slightly reduced under these conditions (Table 1). Because of the steady-state conditions employed, this finding indicates a similar elevation of K influx, an energy-requiring process coupled with Na efflux. This elevation may be secondary to increased diffusion or leak of Na into the cell during DOCA treatment (8) which in turn would stimulate active transport by increasing the cell Na concentration. The finding of elevated aortic Na (Tables 1 and 3) supports this view. The stressing of the active transport system observed when the leak of ions is increased in response to a
reduction in the extracellular Ca concentration indicates that DOCA hypertensives have less transport reserve than do controls. This fact was evidenced by the augmented effect of reduced Ca in lowering K contents of the aortas from hypertensives. More information on the stoichiometry of Na transport in normal and hypertensive rats is needed, however, to rule out a direct effect on active transport and to place these conclusions on a firmer basis.

Increased ion turnover associated with DOCA hypertension would be expected to place an added load on the energy metabolism of the cell. This effect would follow from increased adenosine triphosphate (ATP) utilization for ion transport (32) and increased contractile activity resulting from the resetting of membrane excitability (30). An increase in the metabolic load is thought to play an important role in cardiac hypertrophy (33). It is speculated that increased metabolic activity may provide an early signal for aortic smooth muscle hypertrophy, which, when combined with rising blood pressure, may lead to a shift in the cell’s metabolic pathways to the synthesis of passive elements, e.g., collagen and elastin, in addition to active elements. The greatest change in the weight-length ratio occurred during the period of increasing blood pressure (2–6 weeks) in the present study. Increased amounts of total aortic collagen, elastin, and alkali-soluble protein have been observed during sustained hypertension (34). The vascular smooth muscle cell most likely is the common source of these constituents (35). The present study emphasizes the importance of alterations in membrane properties of vascular smooth muscle to both early and late aortic changes associated with DOCA hypertension.

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A W Jones and R G Hart

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