Temperature Dependence of Ionic Transport and Norepinephrine Stimulation of Rat Aorta During DOCA Hypertension

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SUMMARY Temperature perturbation was used to determine whether increased turnover of Na, K, and Cl at 37°C in aortas from rats made hypertensive with deoxycorticosterone acetate salt treatment (DOCH) reflects an increased number of transport sites that individually maintain relatively normal function. Decreasing temperature reduced the resting effluxes of 42K, 36Cl, and 24Na (active and passive) from control and DOCH in parallel fashion. The slope of the Arrhenius plots (activation energies) and the transition temperatures at which major changes in slope occurred were similar in controls and DOCH. In contrast to the results for resting effluxes, the temperature dependence for the effects of norepinephrine (NE) on contraction and on 42K and 36Cl effluxes in DOCH differed from controls. At 20°C, the responses to NE were either abolished or greatly suppressed in DOCH, as compared to controls, while no significant differences between the two groups were observed at 30°C. These results indicate that alterations in resting 42K, 36Cl, and 24Na effluxes in DOCH may result from an increased number of transport sites in the membranes of vascular smooth muscle. The concept that alterations occurred in the integral components of the membrane is also supported by the observation that increased resting 42K and 36Cl effluxes in DOCH at 37°C persisted in aortas that had undergone cold storage for 2 days before incubation at 37°C. The altered temperature dependence for the effects of NE on DOCH, compared to controls, indicates that the involvement of agonist-receptor-membrane events may be dissociated from the alterations in resting ionic fluxes. (Hypertension 6: 460-467, 1984)

Key Words • vascular smooth muscle • active transport • sodium • potassium • chloride • activation energy • temperature • DOCA hypertension • norepinephrine

ALTERATIONS in several transport systems have been associated with salt-mineralocorticoid hypertension in the rat. Increased passive efflux of 36Cl and 42K occurred in the aorta and femoral arteries from rats made hypertensive either with deoxycorticosterone acetate (DOCA) or aldosterone.1,2 The net exchange of cellular Na and K in tail arteries for extracellular Li also proceeded at a faster rate in DOCA hypertensive rats.3 One important consequence of such alteration would be to increase the leak of ions along their electrochemical gradient, which would lead to altered excitability. In addition to these passive movements, evidence exists for an increased capacity to actively transport 24Na out of aortic smooth muscle4 or to actively transport 86Rb into it.5 Detailed kinetic analyses revealed that the maximal capacity for active transport of Na was elevated, but the parameters associated with the affinity and interaction between sites were not changed.4 These observations led to the hypothesis that mineralocorticoid hypertension is associated with the incorporation of more ion transport sites into the arterial smooth muscle membrane.5,7 The action of plasma digitalis-like factors has been hypothesized to be operative in vivo, to counteract the effect of increased sites for active transport, and to compound the effects of increased leakiness of the cell membrane.8

Membrane supersensitivity to norepinephrine (NE) also occurs during mineralocorticoid hypertension in rats as measured by agonist-induced increases in 42K efflux.9,10 This supersensitivity may extend to several types of agonists including peptides and indicates that a relatively nonspecific supersensitivity develops.11 It is not clear whether the development of drug supersensitivity is closely related to the alteration in resting fluxes, or whether it results from altered receptor syn-
thesis, control, and transduction with membrane channels. Two studies\textsuperscript{10,12} suggest that a close relationship may not exist between alterations in resting fluxes and the development of drug supersensitivity. This possibility was further tested in this investigation.

The studies on resting isotope fluxes and agonist-induced changes during mineralocorticoid hypertension have been, for the most part, conducted at 37°C. Numerous membrane functions including ion transport are modified by altered temperature. Analyses of such temperature dependency (by Arrhenius plots) yield information about the activation energy associated with the function; discontinuities in this function can indicate regions of transition. For instance, in the case of lipid bilayers, such discontinuities are associated with phase transitions between gel and fluid states.\textsuperscript{13} When associated with enzyme activity, acute temperature transitions are thought to reflect a structural alteration of the enzyme.\textsuperscript{14} Relatively few studies have been made of temperature dependence of ion transport in vascular smooth muscle;\textsuperscript{15,16} and no alterations during experimental hypertension have been investigated to our knowledge. Kam and co-workers\textsuperscript{17} made such studies on \textsuperscript{22}Na and \textsuperscript{42}K effluxes from normal rat aortas and aortas from a hibernator (ground squirrel). The effluxes decreased as temperature was lowered from 37°C to 17°C, and an Arrhenius plot of these data was linear. In contrast to the rat, the aorta from ground squirrel exhibited no temperature transition in relation to active Na transport below this temperature.\textsuperscript{17} This adaptation was proposed to be an important factor in the ability of the hibernator to maintain ionic gradients during periods of reduced body temperature.

It was the objective of this study to employ temperature perturbation as a means of determining whether the increased turnover of Na, K, and Cl at 37°C in mineralocorticoid hypertensive rats reflects an increased number of transport sites which individually have relatively normal function. This would be supported by similar activation energies and transition temperatures. On the other hand, alterations in these parameters would provide evidence for altered function of individual sites, perhaps altered membrane lipids, protein structure or modulation. In addition to resting transport processes, it was important to determine whether the temperature dependency of the excitation processes during NE activation exhibits changes that parallel those for resting fluxes.

Preliminary reports of some observations have appeared previously.\textsuperscript{5,18}

Methods

Animals and Tissues

Male Sprague Dawley rats (125–150 g) were unilaterally nephrectomized and divided into control and hypertensive groups. Rats in the hypertensive group (DOCH) had two DOCA pellets (25 mg each) implanted subcutaneously at the time of nephrectomy. A third DOCA pellet was implanted after 2 weeks. All animals were fed standard rat chow. Control rats drank 1% (wt/vol) NaCl, while DOCH drank 1% NaCl plus 0.2% KCl (in order to maintain weight gain).\textsuperscript{1} Systolic blood pressure was measured weekly by a tail cuff technique. Ionic flux experiments were performed 3 to 4 weeks after nephrectomy when all DOCH had developed systolic pressures greater than 180 mm Hg and when controls had systolic pressures less than 130 mm Hg.

All rats were stunned by a blow to the head and exsanguinated by severing the carotid arteries. The thoracic aorta was quickly removed and placed in dissection solution for 90 minutes. During this time the aorta was carefully dissected free of surface fat and loose connective tissue. For isotope studies, the aorta was opened longitudinally and individual pieces were mounted on stainless steel holders prior to experimentation. Tension studies were performed on aortic rings (4 mm wide) mounted on stainless steel loops attached to an isometric force transducer with recordings made on a Grass polygraph. Initial length was established by stretching the ring to 1.5 times its resting diameter. The strips developed near maximal force in response to NE at this length.

Solutions and Isotopes

All solutions were prepared from analytical grade reagents and double-distilled water. The physiological salt solution (PSS) used in these experiments had the following ionic composition (mM): Na, 146.2; K, 5.0; Ca, 2.5; Mg, 1.2; Cl, 143.9; HCO\textsubscript{3}-, 13.5; H\textsubscript{2}PO\textsubscript{4}-, 1.2 and glucose, 11.8. K-free PSS was made by omitting KCl. Dissection solution was identical to PSS except for K = 0 mM and Ca = 0.25 mM. This composition facilitates the removal of endogenous K and reduces the time required to achieve equilibration between the specific activity of \textsuperscript{42}K in the tissue and the isotope solution. These solutions have a pH of 7.4 when gassed with 97% O\textsubscript{2}-3% CO\textsubscript{2} at 37°C. Solutions for the cold storage of rat aortas were slightly modified. The pH of the PSS was adjusted to 7.1 at 1°C by the addition of NaHCO\textsubscript{3}. This provided partial compensation for the increased solubility of CO\textsubscript{2} at 1°C. Solutions containing N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES, 10 mM) as the buffer (HCO\textsubscript{3} omitted) were titrated with NaOH to pH of 7.1 at 1°C. The \textsuperscript{42}K and \textsuperscript{22}Na were obtained from the University of Missouri Research Reactor. Isotope activity was approximately 20 μCi/ml in the final loading solutions. \textsuperscript{38}Cl was obtained as NaCl (ICN) and was added directly to PSS to produce an activity of 1 to 2 μCi/ml.

Cold Storage

One protocol required rat aortas to be stored at 1°C for periods up to 48 hours. After the aorta was excised, the surface fat and loose connective tissue were quickly dissected within approximately 15 minutes. The partially dissected aorta was then placed in a tightly capped test tube that contained either PSS or HEPES solution; the tube was immersed in an ice water bath. A piece of tissue was cut from the aorta on Day 0 (before

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cold storage), on Day 1 of cold storage, and on Day 2 of cold storage. The tissue was trimmed of loose connective tissue and was prepared for isotope flux experiments at 37° C, as described above. Tissue electrolytes were determined on Days 0 and 2 with methods used routinely in this laboratory.4

Isotope Flux Measurements

Tissues were first incubated in radioisotope-containing PSS solution for 3 hours at 37° C. This allowed adequate time for reestablishment of ion gradients and equalization of the isotope specific activity in cellular and extracellular compartments.1, 4 Each tissue was then passed through a series of test tubes containing 5 ml of nonlabeled solution and gassed with 97% O2-3% CO2. The time spent in each tube was predetermined and accurately recorded. At the end of each experiment, the tissue was lightly blotted and weighed. All tissues were extracted into 5 ml of 0.1 N HNO3 solution, and later neutralized with NaOH. A weighed sample of the radioisotope-loading solution was treated similarly. Samples of 42K were counted in a gamma counter. Samples of 36Cl and 24Na were mixed with 10 ml of liquid scintillation cocktail (Research Products, Inc., Mt. Prospect, Illinois), and beta emission was counted.

Data processing was by computer. The program made corrections for isotope decay (24Na and 36K) and background counts. The corrected counts for tissue and washout tubes were sequentially added in reverse order, which allowed the determination of the counts remaining in the tissue during each washout period. This value was converted to the fraction counts remaining by dividing by the tissue counts at time zero. Rate constants, k, were calculated by dividing the tissue counts washed out per minute by the average counts in the tissue during the washout period, and are expressed as fractional loss per minute.

Temperature Studies

42K Efflux

In one group of experiments, the rate constants were determined at each of several temperatures. This was accomplished by changing the temperature of the washout solution every 40 minutes during the efflux. The order of exposure to the various temperatures was 37°, 30°, 20°, 10°, 5°, and 1° C. The washout during the last 10-minute period at each temperature was used to compute the rate constant for that temperature. This slope analysis yielded rate constants that were equivalent to those derived from the time required for the fraction of tissue counts to decline to 1/e of its initial value.15 The rate of the K-dependent efflux was reported to be equivalent to the ouabain-dependent rate constant and associated efflux in rat aorta.4

36Cl Efflux

The 36Cl turnover was determined at the desired temperatures in a manner similar to that described for 42K efflux. The only differences in protocol were: 1) the last 25 minutes in the isotope-containing solution were at the temperature to be tested during the washout; 2) NE was applied for 10 minutes following 15 minutes of washout; and 3) only one temperature was used per efflux curve because of the small number of counts remaining after 25 minutes of washout. The 36Cl rate constant was defined as the reciprocal of the time required for the slow fraction to fall to 1/e of its initial value.15 The cellular fraction was estimated to be the fraction of total tissue counts remaining after a 1-minute washout, which is the point where a sharp transition occurs between the fast and slow components.1 This slow component exhibits sensitivity to metabolic inhibitors, anion substitution, and NE, which is consistent with a component that is transported across the cell membrane. Responses to NE were calculated from the difference between the maximal rate in the presence of the drug and the rate for the washout period immediately preceding the drug.

24Na Efflux

Measurement of 24Na efflux was complicated by the high ratio of extracellular to cellular sodium. It was essential to clear the extracellular space (ECS) of labeled sodium and to maintain the cellular 24Na at the same time. This was accomplished by beginning the isotope washout at 1°C, effectively inhibiting active sodium extrusion. After time was allowed for the ECS to be cleared of 24Na, the tissue was then transferred to a bath at the desired temperature.

The final 5 minutes of loading in 24Na were performed at 1°C for all tissues. The initial 10 minutes of washout were conducted at 1°C followed by a washout at one of the experimental temperatures. The 24Na rate constant was defined as the reciprocal of the time required for the fraction of tissue counts to decline to 1/e of its value when the incubation temperature was increased.4 15 Paired tissues were studied for all sodium efflux experiments. Both tissues were loaded with 24Na in PSS with [K] = 5 mM. One tissue was washed out in PSS with [K] = 5 mM, and the other in PSS with [K] = 0 mM. Removal of extracellular potassium inhibits active sodium extrusion, allowing the determination of K-independent (passive) sodium rate. The K-dependent (active) rate, which is operationally defined to be active transport, was calculated as the difference between total and K-independent sodium rate constants. The rate of the K-dependent efflux was reported to be equivalent to the ouabain-dependent rate constant and associated efflux in rat aorta.4
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Statistics
Data are presented as means plus or minus the standard error of the mean (SEM). Student's t test was used to estimate significance. The level of significance was chosen as \( p < 0.05 \). Lines of best fit were determined by the least-squares regression technique.

Results
Temperature Dependence of Ionic Fluxes
\(^{42}\)K Efflux

The temperature dependence of \(^{42}\)K efflux (Figure 1) exhibited two important properties: \(^{42}\)K was washed out of the DOCH at a much faster rate than from the control aortas, and the slope of the washout curve was reduced as the temperature was lowered from 37° to 10° C, which signified a slowing of the \(^{42}\)K turnover with decreased temperature. Rate constants for aortas from control and DOCH are presented in Table 1. The significant elevation in \(^{42}\)K rate constants exhibited by DOCH aortas at 37° C persisted to 10° C, but the slightly higher values at 5° and 1° C were not statistically significant. The rate constants at the end of the experiment (37° C) were within 8% of those at the beginning, which confirmed monoexponential behavior of the efflux.

An Arrhenius plot of the \(^{42}\)K data is shown in Figure 2. This plot is based on the linearized form of the Arrhenius equation: \( \ln k = -\frac{E_a}{RT} + \text{constant} \), where \( k \) = rate constant; \( R \) = gas constant; \( T \) = temperature (°K); and \( E_a \) = energy of activation Kcal/mole. A plot of \( \ln k \) vs \( 1/T \) (°K\(^{-1}\)) allows the calculation of \( E_a \) from the slope. The Arrhenius plot will be linear for a homogeneous process, while a discontinuity in the plot represents a change in the activation energy that may reflect a structural alteration in the system.\(^14\) The \( E_a \) calculated from the slope, as determined by least-squares linear regression (37°-10° C) was 9.0 Kcal/mole for the control and 9.5 Kcal/mole for the DOCH. The slopes of the two regression lines did not differ significantly (\( p > 0.40 \)). A discontinuity in the Arrhenius plot of \(^{42}\)K rate occurred at 10° C for both control and DOCH.

Table 1. Effects of Temperature on \(^{42}\)K and \(^{36}\)Cl Rate Constants (min\(^{-1}\)) in Aortas from Control and DOCH

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>(^{42})K Control (n = 7)</th>
<th>(^{42})K DOCH (n = 9)</th>
<th>(^{36})Cl Control (n = 7)</th>
<th>(^{36})Cl DOCH (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>0.0092 ± 0.0003</td>
<td>0.0164 ± 0.0007(^{\dagger})</td>
<td>0.119 ± 0.006</td>
<td>0.151 ± 0.014(^*)</td>
</tr>
<tr>
<td>30</td>
<td>0.0056 ± 0.0002</td>
<td>0.0100 ± 0.0005(^{\dagger})</td>
<td>0.044 ± 0.004</td>
<td>0.070 ± 0.006(^{\dagger})</td>
</tr>
<tr>
<td>20</td>
<td>0.0030 ± 0.0002</td>
<td>0.0058 ± 0.0007(^{\dagger})</td>
<td>0.018 ± 0.002</td>
<td>0.029 ± 0.001(^{\dagger})</td>
</tr>
<tr>
<td>10</td>
<td>0.0023 ± 0.0002</td>
<td>0.0037 ± 0.0003(^{\dagger})</td>
<td>0.009 ± 0.001</td>
<td>0.014 ± 0.002(^*)</td>
</tr>
<tr>
<td>5</td>
<td>0.0033 ± 0.0004</td>
<td>0.0042 ± 0.0004</td>
<td>0.011 ± 0.001</td>
<td>0.016 ± 0.003</td>
</tr>
<tr>
<td>1</td>
<td>0.0036 ± 0.0003</td>
<td>0.0045 ± 0.0006</td>
<td>0.014 ± 0.002</td>
<td>0.020 ± 0.003</td>
</tr>
<tr>
<td>37</td>
<td>0.0091 ± 0.0003</td>
<td>0.0177 ± 0.0013(^{\dagger})</td>
<td>0.119 ± 0.006</td>
<td>0.151 ± 0.014(^*)</td>
</tr>
</tbody>
</table>

Data are means ± SEM. DOCH = deoxycorticosterone-acetate-salt treated hypertensive rat.

\(^\dagger p < 0.05\) for comparisons between control and DOCH.

\(^* p < 0.05\) for comparisons between control and DOCH.
$^{36}\text{Cl Efflux}$

The temperature dependence of $^{36}\text{Cl}$ turnover resembled that observed for potassium. The rate constants (Table 1) decreased steadily as temperature was lowered from 37° to 10° C, while below 10° C the rates increased slightly. Chloride turnover was significantly increased in aorta from DOCH at 10° C or above. The Arrhenius plot (Figure 3) was relatively linear between 37° and 10° C, and the slopes of the control and DOCH plots were not significantly different ($p > 0.20$). The $E_a$ calculated from the regression line (least-squares technique between 37°–10° C) was 16.5 Kcal/mole for control and 15.3 Kcal/mole for DOCH. An inflection point occurred in the Arrhenius plot at 10° C for each group.

$^{24}\text{Na Efflux}$

Total sodium efflux consisted of both active and passive elements. Active extrusion of sodium was inhibited by removal of extracellular potassium, which allowed a more direct measurement of passive components. Passive $^{24}\text{Na}$ efflux was significantly elevated in DOCH at temperatures of 15° C or greater (Table 2). This component was approximately equal to the active $^{24}\text{Na}$ efflux at temperatures between 37° and 15° C in both experimental groups. The Arrhenius analyses of the rate constants associated with K-independent sodium efflux yielded slopes for the linear regression (37°–15° C) that were not significantly different for control and DOCH ($p > 0.20$). The $E_a$ was 12.8 Kcal/mole for controls and 13.2 Kcal/mole for DOCH.

**Table 2. Effects of Temperature on Rate Constants (min$^{-1}$) for K-independent and K-dependent $^{24}\text{Na}$ Efflux in Aortas from Control and DOCH**

<table>
<thead>
<tr>
<th>Temperature ($^\circ$ C)</th>
<th>K-independent</th>
<th>K-dependent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 6)</td>
<td>DOCH (n = 9)</td>
</tr>
<tr>
<td>37</td>
<td>0.106 ± 0.007</td>
<td>0.145 ± 0.010*</td>
</tr>
<tr>
<td>30</td>
<td>0.084 ± 0.003</td>
<td>0.113 ± 0.004†</td>
</tr>
<tr>
<td>20</td>
<td>0.036 ± 0.003</td>
<td>0.047 ± 0.002*</td>
</tr>
<tr>
<td>15</td>
<td>0.019 ± 0.001</td>
<td>0.039 ± 0.005†</td>
</tr>
<tr>
<td>10</td>
<td>0.016 ± 0.001</td>
<td>0.018 ± 0.001</td>
</tr>
<tr>
<td>1</td>
<td>0.013 ± 0.001</td>
<td>0.014 ± 0.001</td>
</tr>
</tbody>
</table>

Data are means ± SEM. See Table 1 for abbreviations.

* $p < 0.05$ for comparisons between controls and DOCH.
† $p < 0.005$ for comparisons between control and DOCH.
The active transport of $^{24}$Na was significantly greater in DOCH than in controls for temperatures between 15° and 37° C (Table 2). Arrhenius plots for K-dependent sodium efflux (Figure 4) had similar $E_a$ for control and DOCH (12.7 and 13.4 Kcal/mole) between 37° and 15° C. A dramatic increase in $E_a$ occurred between 15° and 10° C for both groups, with values of 41.9 Kcal/mole for controls and 40.7 Kcal/mole for DOCH. Functionally, this increase in $E_a$ was associated with about 95% inhibition of the Na-K pump at 10°. The variability in the $^{24}$Na efflux technique was such that small changes in rate constants could not be accurately measured at temperatures below 10° C.

**Response to Norepinephrine**

The effect of NE on $^{42}$K turnover, $^{36}$Cl turnover, and tension was examined in aortas from control and DOCH at temperatures ranging from 37° to 10° C (Table 3). Reduction of the incubation temperature from 37° to 30° C had little effect on the NE stimulation of ion turnover and contraction in aortas from either group. Further temperature reduction to 20° C abolished the contractile response to NE in aortas from DOCH, while the controls continued to respond. This result was also reflected in the effects on isotope effluxes (Table 3). Aortas from control rats failed to respond to NE only when the incubation temperature was lowered below 10° C. The Arrhenius plot of the NE effect on $^{42}$K turnover (Figure 5) showed a large increase in slope for DOCH between 30° and 20° C, whereas this abrupt change in slope or transition occurs between 20° and 10° C for controls. In sharp contrast to the effects on relaxing ion turnover, the temperature dependence of NE excitation in DOCH exhibited significant differences in comparison to controls.

**Cold Stored Tissue**

Aortas from control and DOCH were stored at 1° C for up to 48 hours before $^{42}$K and $^{36}$Cl effluxes were measured. It was reasoned that if a humoral factor were responsible for the increased ion turnover in DOCH aorta, then that factor would be washed out of the tissue during prolonged storage. There were no differences between tissues stored either in HCO$_3$ or HEPES ($p > 0.40$) on any given day; therefore, data from HCO$_3$ and HEPES stored tissue were combined. As shown in Table 4, the rate constants for $^{42}$K were elevated in DOCH on all 3 days. There was a trend for the $^{42}$K rate constants within each group to increase with cold storage ($p < 0.025$). The $^{36}$Cl rate constants also remained elevated in DOCH after 1 and 2 days of cold storage. No significant changes in $^{36}$Cl efflux were associated with the duration of cold storage. The persistence of higher rate constants in DOCH after 48 hours of cold storage suggested that there was an inherent difference in the membrane composition of DOCH, rather than an acutely active humoral factor.

The viability of the tissues after 48 hours of storage at 1° C was checked by testing the ability of the cells to reaccumulate potassium. Potassium content of rat aortas was determined photometrically on Days 0 and 2. Control aortas had a potassium content of 139 ± 6

![Figure 5. Log (Δk) vs reciprocal temperature plotted as in Figure 2. The Δk represents the increase in $^{42}$K rate constant following norepinephrine (NE) (6 $\times$ 10$^{-6}$ M). Data are means ± sem for control (○) n = 6 and DOCH (●) n = 6 rats.](image)
mEq/g dry solid on Day 0, and 100 ± 4 mEq/g dry solid on Day 2. The potassium content of DOCH was 167 ± 2 mEq/g dry solid and 140 ± 5 mEq/g dry solid on Days 0 and 2 respectively. Since tissues were able to accumulate approximately 75% of their original K after 48 hours of cold storage, most cells appeared viable.

Discussion

Temperature Dependence of Ionic Fluxes

The increased effluxes of $^{42}$K, $^{36}$Cl, and $^{24}$Na associated with DOCA hypertension at 37° C persisted at reduced temperatures. Moreover, the slopes of the Arrhenius plots and the transition temperatures were similar in both groups. These observations support the conclusion that an increase occurs in the capacity of the sites available for transport. A corollary to this conclusion is that the individual sites appear to be operating similarly in controls and DOCH. The temperature dependence of the control fluxes of $^{42}$K and $^{24}$Na is in good agreement with the findings of an earlier study. For instance, the $E_o$ and transition temperatures for $^{42}$K and K-dependent (active) Na transport are almost identical in the two studies. Overall, the parameters related to the Arrhenius plots are reproducible.

We concluded that major changes (greater than 30%) associated with hypertension should be demonstrable by this approach, but we did not observe such effects. These results confirm and extend previous conclusions concerning an increased number of sites for active Na transport in the membranes of vascular smooth muscle from DOCH. It was observed in those studies that the maximal capacity for active Na transport at 37° C was increased in DOCH, while the parameters associated with the affinity and site-to-site interaction were not significantly altered.

Friedman et al. have suggested that increased synthesis of membrane proteins may be a significant factor in altering ion metabolism of vascular smooth muscle during salt-mineralocorticoid treatment. Recent investigations have confirmed the presence of high affinity binding sites for mineralocorticoids in rat and rabbit arteries. Kornel has hypothesized that the altered movements of aortic Na associated with DOCA treatment of rabbits result from the direct cellular effects of steroid-receptor-nuclear activation. At this time, the molecular mechanisms that underly altered ion transport and excitation during mineralocorticoid hypertension are not well understood, but hormonal regulation of synthesis of membrane proteins in vascular smooth muscle appears to be of fundamental importance.

Response to Norepinephrine

The temperature dependence of NE-induced changes in ionic fluxes ($\Delta k$) and force development differed in controls and DOCH. This difference was readily seen at 20° C where the NE-induced changes in $^{42}$K efflux and contraction were 40% of the response at 37° C, and the NE effects on $^{36}$Cl were maintained at normal levels in the control series. In sharp contrast, the responses in the DOCH at 20° C were 0 to 25% of the level at 37° C. This loss of responsiveness in DOCH is associated with apparent differences in transition temperatures and slope of the Arrhenius plots. For instance, a transition temperature near 30° C was observed in the $E_o$ for $^{42}$K efflux from DOCH, while the temperature dependence in controls exhibited relatively linear behavior between 37° to 20° C. Force development was maintained equally well in both groups at 30° C, but the DOCH failed to respond to NE at 20° C under conditions in which controls developed 40% of the force at 37° C. This difference is consistent with an increased slope or $E_o$ for the contractile response in DOCH. The $E_o$ for $^{36}$Cl efflux from controls was maintained over the 37° to 20° C range, while DOCH exhibited a progressive reduction. Given the complexities of the excitation-contraction coupling and force development mechanisms in vascular smooth muscle, it is not surprising that some differences were observed in the temperature dependence of individual components ($^{42}$K, $^{36}$Cl, force). Of importance for this study is that each component studied in DOCH exhibited either a shift in transition temperature or slope ($E_o$). These observations indicate that changes associated with excitation and contraction in DOCH may be dissociated from alterations in resting (non-stimulated) ionic fluxes. This tentative conclusion is consistent with the observation that a fully developed supersensitivity to NE in the rat aorta occurs after 1 week of treatment with aldosterone, well before the maximal changes occurred in resting $^{42}$K effluxes. The supersensitivity to catecholamines that develops after denervation of the vas deferens in guinea pig also occurs without measurable changes in resting $^{42}$K effluxes. Further studies are required to define more precisely the relationship between changes in receptor-coupled membrane fluxes and resting fluxes associated with mineralocorticoid-salt hypertension. At this time, the possibility arises that these events may occur independently.

Force developed by rat aorta in response to NE has been measured at reduced temperature by Peiper et al., who observed that the force decreased as tem-
perature was lowered and that the relationship was linear over the range of 33° to 15° C. The maximal NE contraction at 15° C was only 3% of that obtained at 37° C. These findings are similar to the control data presented in the current investigation. Causes of the decreased contractile response to NE at low temperature are not well established, however. The maximum force developed by glycerinated fibers from hog carotid artery (stimulus = 20 mM adenosine triphosphate (ATP) is independent of temperature (10°-45° C), although the rate of tension development is diminished at low temperature.16 This observation suggests that contractile proteins are not the limiting step for reduced force development under these conditions. Rather, the agonist-receptor-membrane events appear to be limiting at reduced temperature. More detailed experiments will be required, however, to determine which steps are most involved.

Cold Stored Tissue

The ability of the controls to reaccumulate tissue K to near normal levels after 48 hours in cold storage confirms an earlier study.25 The slight reduction in cellular content of K may be related to a similar reduction in aortic cell water,25 perhaps the result of the loss of some cellular material. The increase in the rate constants for 42K on Day 2 compared with freshly dissected arteries may also be contributory. On the other hand, the rate constants for 36Cl did not change significantly with cold storage. It appears the tissues were adequately preserved to test the hypothesis that changes in resting fluxes from DOCH result from inherent changes in membrane composition and are less likely to result from the direct action of some plasma component, such as a digitalis-like humoral factor that may be operative for several hours.8 If such a compound were present in freshly excised tissue, it would likely be washed out by 48 hours in vitro. The significant elevation in the 42K and 36Cl effluxes after 24 and 48 hours indicates that the increased transport by DOCH aortas reflect intrinsic membrane transport differences more than the action of an extrinsic circulatory hormone. This does not rule out, however, that chronic in vivo exposure to such factors may induce changes in the composition of the membrane.

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