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The Effect of Norepinephrine on Aortic \( ^{42} \text{K} \) Turnover during Deoxycorticosterone Acetate Hypertension and Antihypertensive Therapy in the Rat

ALLAN W. JONES, PAUL D. SANDER, AND DONALD L. KAMPSCHMIDT

SUMMARY We studied the effects of norepinephrine on \( ^{42} \text{K} \) turnover in aorta isolated from rats. The rats were given saline to drink and were made hypertensive by injections of deoxycorticosterone acetate (DOC). Other groups of rats received in addition either 6-hydroxydopamine (6-OH-DA) or a regimen of antihypertensives (Anti-Hy) consisting of reserpine, hydrochlorothiazide, and hydralazine. The weight, length, wall thickness, and circumference of the aorta also were measured. DOC hypertension was associated with increased \( ^{42} \text{K} \) turnover (rate constant for DOC = 0.0164 ± 0.0009 vs. 0.0090 ± 0.0002 min\(^{-1}\) in controls). The responses of \( ^{42} \text{K} \) turnover to low doses of norepinephrine (NE) were increased in DOC with an ED\(_{50}\) of 3.5 ± 0.8 \times 10^{-9} \text{ M} vs. 2.7 ± 0.5 \times 10^{-8} \text{ M} in controls. The aortic weight, weight/length, and wall thickness were also increased. Rats treated with DOC plus 6-OH-DA had lower blood pressure and smaller changes in aortic dimensions; however \( ^{42} \text{K} \) turnover and response to NE were similar to those of the DOC group. The Anti-Hy group exhibited only small increases in \( ^{42} \text{K} \) turnover and aortic dimensions when compared to controls. It is concluded that DOC hypertension is associated with increased response of \( ^{42} \text{K} \) turnover to NE which in turn may contribute to increased responses reported for contraction. The Anti-Hy regimen was more effective than 6-OH-DA in reducing the increased \( ^{42} \text{K} \) turnover and response to NE associated with DOC hypertension.

INCREASED turnover of ions in vascular smooth muscle\(^1\) has been associated with hypertension induced by deoxycorticosterone (DOC) + salt treatment of rats. An increased contractile response to low doses of catecholamines by isolated arteries\(^4\) and in perfused vascular beds\(^7\) has also been associated with DOC hypertension. Such increased contractile responses may result from altered function in one or more of the many steps involved with excitation-contraction coupling. Isotope exchange techniques offer a means for separating membrane from intracellular events as potential sources for contractile changes, in that the slow turnover of small ions such as K\(^+\) and Cl\(^-\) is thought to be limited by the surface membrane.\(^2\) Norepinephrine (NE) has been observed to increase aortic \( ^{42} \text{K} \) turnover in a dose-dependent manner.\(^8\) Furthermore, increased response to NE was found to be associated with spontaneous hypertension in the rat.\(^6\)\(^9\) It therefore was of interest to determine whether an increased response of aortic \( ^{42} \text{K} \) turnover to NE was associated with the shift in contractile responses during DOC hypertension.

The role of the sympathetic nervous system in the development of DOC hypertension is of special importance in light of increased responses to catecholamines. One approach taken to this problem was to treat rats with 6-hydroxydopamine (6-OH-DA), an agent which is thought to produce a selective destruction of adrenergic nerve terminals.\(^10\) Treatment with 6-OH-DA was observed to retard but not prevent the development of DOC hypertension.\(^11\)\(^12\) Another approach which employed a combination of antihypertensive drugs (reserpine, hydrochlorothiazide and hydralazine) reversed the pressure rise in DOC hypertension; however, the increased response to NE associated with DOC was reduced but not reversed.\(^13\)

The primary objectives of this study were to determine (1) whether the response of \( ^{42} \text{K} \) turnover to norepinephrine was increased in DOC hypertension, (2) whether the vascular changes are subject to modification by chronic treatment with 6-OH-DA, and (3) the effect of a broad-based antihypertensive regimen on aortic dimensions and \( ^{42} \text{K} \) turnover in rats treated with DOC-saline.

Methods

ANIMAL AND TISSUE PREPARATION

The left kidney was removed from anesthetized male Wistar rats weighing 200 g. Rats in the control group were given saline (1% wt/vol) to drink; and saline was supplemented with KCl (0.2% wt/vol) for those receiving DOC. Rats in the DOC group were injected twice each week for 6–7 weeks with DOC, 6 mg in sesame oil. The antihypertensive regimen (Anti-Hy) and treatment with 6-OH-DA were initiated 1–5 days postoperatively. The Anti-Hy regimen was similar to that of Finch\(^13\) and consisted of hydrochlorothiazide (Ciba), 250 mg/liter, reserpine (Sigma), 5 mg/liter, and hydralazine (Sigma), 100 mg/liter added to the drinking saline and given ad lib. The rats consumed 60–100 ml/day. The dose schedule for 6-OH-DA was that of de Champlain and van Ameringen.\(^11\) The 6-OH-dopamine hydrobromide (Sigma) was dissolved in 1% NaCl containing 1% ascorbic acid and gassed with nitrogen.
Injections of 100 mg/kg were given intraperitoneally at weekly intervals until the day before the experiment. Rats were killed by a blow to the head. The thoracic aorta was removed and placed in a dissection solution. Loose connective tissue was trimmed and a ring cut for dimensional analyses. The thoracic aorta (1st intercostal to superior celiac artery) was cut axially, its length was measured, and it was mounted on a stainless steel holder.

**SOLUTIONS**

The normal physiologic solution had the following millimolar composition: Na⁺, 146.2; K⁺, 5.0; Mg²⁺, 1.2; Ca²⁺, 2.5; Cl⁻, 143.9; HCO₃⁻, 13.5; H₂PO₄⁻, 1.2; and glucose, 5.7. Solutions were gassed with 97% O₂-3% CO₂ at 37°C which resulted in a pH of 7.4. The dissection solution was K⁺-free and contained 0.2 mM Ca²⁺, which allowed reversible depletion of tissue K⁺ during the 1/2-hour dissection. This was necessary to make certain that the specific activity of cellular K⁺ was within a few percent of that in the isotope solution after incubation. Solutions containing norepinephrine (Winthrop) were made by serial dilution (Statham P23AC transducer) at which pulsations (Narco) had been previously trained to rest quietly in a plastic restraining case that was gently heated. The cuff pressure (Statham P23AC transducer) at which pulsations (Narco transducer) first reappeared was taken as systolic pressure and expressed in mm Hg. Values from at least three observations made during quiet periods were averaged.

**ISOPO TECHNIQUES**

The procedures have been previously used and evaluated. Briefly, the aorta was incubated for 3 hours in a solution containing ⁴²K (University of Missouri nuclear reactor). After a 2-second rinse, the aorta was moved through a series of tubes containing nonradioactive solution to determine steady state turnover. The tissue then was passed through three tubes containing NE for a 10-minute exposure. This was followed by two 10-minute washes before exposure to the next dose. A gamma well was used to count ⁴²K. The washout curves were calculated by sequentially adding the tissue and tube counts in reverse order and normalizing them in terms of fraction of initial activity. A digital computer was used to process the data. The fraction exchanged per minute for each washout period also was computed. Under steady state conditions this represents the rate constant, k (min⁻¹). Values for the 30- to 40-minute period (just before the first NE exposure) were used for statistical comparisons. Dose-response relations were derived from standard normalizing procedures used for the study of drug supersensitivity. The response to a given dose of NE, Δk, was taken as the difference between the highest rate constant in the presence of the agonist and the rate constant for the wash period just before exposure to NE. The maximal response, Δk_max, was taken as the response to a supramaximal dose of 6 x 10⁻⁴ M. The individual responses, Δk, were normalized in terms of Δk_max for each aorta and represented as percent. The median effective dose, ED₅₀, was determined for each aorta by linear interpolation between the log dose just below and just above the 50% response. Statistical comparisons of ED₅₀ used the arithmetic mean.

The cellular pool of K⁺ was estimated from the counts of ²K TURNOVER AND ANTIHYPERTENSIVES/JOHNSON ET AL. 257

Results

**RESULTS**

The steady state turnover of ⁴²K in the aorta of DOC-treated rats was higher than that of controls (Fig. 1 and Table 1), confirming earlier observations. Exposure to NE increased ⁴²K turnover in a dose-dependent manner as shown in Figure 1. The lowest dose induced only a transient increase in both groups. The DOC group, however, exhibited a consistent response to 6 x 10⁻⁴ M that was 4 times greater than that of controls. The maximum change, Δk_max, induced by 6 x 10⁻⁴ M NE was slightly higher for the control group (Fig. 2). The shift in the dose-response curve was not parallel. The controls exhibited a greater slope for high doses.
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no apparent rela-

tion was seen between body weight and aortic changes. Anti-Hy treatment appears in Table 1. The rats from the

controls receiving Anti-Hy and about 10-fold

controls. The ED 50 was increased 2-fold

with reduced turnover of 42K in the DOC group but not in

controls. The ED 50 was increased 2-fold (P < 0.005) in controls receiving Anti-Hy and about 10-fold (P < 0.001) in DOC rats.

A more detailed comparison of the effects of DOC and Anti-Hy treatment appears in Table 1. The rats from the

four groups had similar body weights. No apparent rela-
tion was seen between body weight and aortic changes.

Threshold between the presence of 6-OH-DA and Anti-Hy; however, the magni-

tude of this change was significantly reduced (P < 0.01).

Treatment with 6-OH-DA had little effect on the turnover

of 42K and the ED 50 for NE in both control and DOC rats. The effect was greatest in rats receiving DOC. The aortic

circumference was similar for all groups. The

changes in these parameters between controls and DOC + Anti-Hy, however, was not significant. The aortic circumference was similar for all groups. The Anti-Hy regimen also reduced, but did not prevent, the increase in 42K turnover associated with DOC hypertension. The Anti-Hy regimen also lowered blood pressure as shown in Figure 3.

The only significant effects on these measurements were significantly reduced when Anti-Hy accompanied the DOC treatment. The Anti-Hy regimen was not totally effective in preventing the changes in aortic dimensions, as indicated by the significant differences in these parameters between controls and DOC + Anti-Hy. The difference in blood pressure between controls and DOC + Anti-Hy, however, was not significant.

The effect was greatest in rats receiving DOC. The aortic

circumference was similar for all groups. The

changes in these parameters between controls and DOC + Anti-Hy, however, was not significant. The aortic circumference was similar for all groups. The Anti-Hy regimen also reduced, but did not prevent, the increase in 42K turnover associated with DOC hypertension. The Anti-Hy regimen also lowered blood pressure as shown in Figure 3.

Figure 1. Effects of norepinephrine (NE) on the fraction of aortic 42K exchanged per minute from controls (●) and deoxycorticosterone (DOC) hypertensives (○). Points are joined by straight lines and a representative standard error (SEM) is indicated by vertical bars. The horizontal bars show the period of exposure to NE at the indicated doses.

**ANTIHYPTENSIVE TREATMENT**

Both treatment with 6-OH-DA and the antihyperten-
sive regimen lowered blood pressure as shown in Figure 3. The effect was greatest in rats receiving DOC. The aortic weight-length ratio was elevated in DOC hypertensive rats (Fig. 3, Table 1). An elevation was still manifest in the DOC + Anti-Hy regimen was associated with reduced turnover of 42K in the DOC group but not in controls. The ED 50 was increased 2-fold (P < 0.005) in controls receiving Anti-Hy and about 10-fold (P < 0.001) in DOC rats.

A more detailed comparison of the effects of DOC and Anti-Hy treatment appears in Table 1. The rats from the four groups had similar body weights. No apparent relation was seen between body weight and aortic changes.

Figure 2. Dose-response relation for control (●) and deoxycorticosterone (DOC) hypertensives (○) ± SEM. The points are joined by straight lines. The ED 50 values are indicated for control (○) and DOC (●) ± SEM.

DOC treatment was associated with increased aortic weight, weight-length ratio, and wall thickness. All of these measurements were significantly reduced when Anti-Hy accompanied the DOC treatment. The Anti-Hy regimen was not totally effective in preventing the changes in aortic dimensions, as indicated by the significant differences in these parameters between controls and DOC + Anti-Hy. The difference in blood pressure between controls and DOC + Anti-Hy, however, was not significant. The aortic circumference was similar for all groups. The Anti-Hy regimen also reduced, but did not prevent, the increase in 42K turnover associated with DOC hypertension. The increased ED 50 for NE associated with Anti-Hy was not related to effects on Δkmax. The only significant effects on the controls (no DOC) of Anti-Hy was reduced pressure and increased ED 50 (P < 0.005).

**Table 1. Effects of Deoxycorticosterone (DOC) and Antihypertensive Treatment (Anti-Hy) on Aortic Dimensions and 42K Turnover**

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood press. (mm Hg)</th>
<th>Body wt (g)</th>
<th>Aortic wt (mg)</th>
<th>Weight/ length (mg/cm)</th>
<th>Wall thickness (μm)</th>
<th>Circumference (cm)</th>
<th>42K turnover (mmol/kg)</th>
<th>k (min⁻¹)</th>
<th>NE Δkmax (min⁻¹)</th>
<th>NE ED 50 (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8 ±4</td>
<td>135 ±7</td>
<td>336 ± 2</td>
<td>9.7 ± 0.2</td>
<td>142 ± 4</td>
<td>5.5 ± 1.2</td>
<td>32.8 ± 0.009</td>
<td>0.0151</td>
<td>2.7 ± 10⁻⁴</td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>7 ±6</td>
<td>225* ±6</td>
<td>47.1* ± 3</td>
<td>13.0* ± 0.3</td>
<td>190* ± 5.7</td>
<td>5.7 ± 0.2</td>
<td>44.9* ± 0.0164*</td>
<td>0.0125</td>
<td>3.5 ± 10⁻⁴</td>
<td></td>
</tr>
<tr>
<td>Control + Anti-</td>
<td>8 ±10</td>
<td>108 ±7</td>
<td>31.3 ± 3</td>
<td>9.1 ± 1</td>
<td>140 ± 6.0</td>
<td>3.17 ± 0.0009</td>
<td>0.0191 ± 6.2 ± 10⁻⁴</td>
<td>0.0141</td>
<td>4.0 ± 10⁻⁴</td>
<td></td>
</tr>
<tr>
<td>Hy</td>
<td>±3 ±8</td>
<td>146 ±8</td>
<td>39.6* ± 1</td>
<td>11.0* ± 0.3</td>
<td>164* ± 6.0</td>
<td>3.0 ± 1.1</td>
<td>40.6* ± 0.0117*</td>
<td>0.0141</td>
<td>4.0 ± 10⁻⁴</td>
<td></td>
</tr>
<tr>
<td>DOC + Anti-Hy</td>
<td>10 ±3</td>
<td>338 ±15</td>
<td>39.6* ± 1</td>
<td>11.0* ± 0.3</td>
<td>164* ± 6.0</td>
<td>3.0 ± 1.1</td>
<td>40.6* ± 0.0117*</td>
<td>0.0141</td>
<td>4.0 ± 10⁻⁴</td>
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<tr>
<td>t-test</td>
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</tr>
<tr>
<td>Control vs.</td>
<td>NS</td>
<td>NS</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td>P &lt; 0.01</td>
<td>P = 0.01</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>DOC + Anti-Hy</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td>P &lt; 0.005</td>
<td>NS</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM; n = number of rats; NS = not significant.

* P < 0.001

† P < 0.025 for control vs. DOC; control + Anti-Hy vs. DOC + Anti-Hy.
**Discussion**

Altered membrane transport of K⁺ and Cl⁻ during DOC + saline hypertension can now be associated with increased response of ⁴²K turnover to NE. This is indicated by (1) an increased response to a low dose of NE (6 × 10⁻¹⁰ M) and (2) a 7-fold shift in ED₅₀. The shift in ED₅₀, although in the same direction, is greater than that reported by Holloway and Bohr for epinephrine on isolated femoral arteries. Further work is needed to determine whether the difference in agonist and arterial site might account for the quantitative difference. The finding of increased K⁺ turnover and ED₅₀ for norepinephrine (NE) increases contractile activity for a given level of NE or sympathetic activity. Such speculation will need confirmatory electrophysiological evidence, and does not rule out alteration of other cellular events, e.g., calcium storage and release.

Reducing sympathetic activity by chronic administration of 6-OH-DA did not greatly influence the increased ⁴²K turnover and response to NE during DOC treatment. There was some reduction in blood pressure and aortic weight-length ratio, and this indicates that the change in aortic dimensions is in part related to pressure. The smooth muscle membrane effects, however, may be more closely related to DOC + saline treatment than to increased blood pressure. An intact sympathetic innervation does not appear to be a requirement for elevation of blood pressure during DOC + saline treatment. This has been explained in terms of incomplete destruction of sympathetic nerves by 6-OH-DA in arteries or hyperactivity of the adrenal medulla. Increased sensitivity of vascular smooth muscle to catecholamines may well play an important role. For example, the response of controls to 6 × 10⁻⁹ M NE (Fig. 1) was achieved with a dose of 6 × 10⁻¹⁰ M in DOC-treated rats. If a change of similar magnitude occurs in small vessels, the residual sympathetic activity and adrenal medullary secretion after 6-OH-DA could be sufficient to maintain blood pressure.

The application of a broadly based regimen of antihypertensive therapy was more effective than 6-OH-DA in preventing increased ⁴²K turnover and response to NE associated with DOC + saline treatment. The reduced response does not agree with the findings of Finch that an increased responsiveness of perfused mesenteric arteries persisted after such treatment. Although the antihypertensive regimens were the same, the time of administration was different. We first gave the antihypertensive agents at the time of nephrectomy and initial DOC treatment and before hypertension was established, whereas Finch started the regimen after DOC hypertension (and increased responses) had been established. This observation may have a practical application in developing strategies for treating hypertension. Certain alterations in smooth muscle may be prevented by early treatment but not readily reversed after hypertension has become fully established. The antihypertensive regimen also was effective in reducing aortic size as measured by three parameters; aortic weight, weight-length ratio, and wall thickness. However, some increase in aortic size was present despite only a small rise in systolic blood pressure. Factors in addition to blood pressure may operate to increase the size of the aortic wall during DOC + saline treatment. It is not known which of the antihypertensive agents was most effective or whether there was an important interaction between the treatments. Additional studies with various combinations, dose schedules, and hypertensive models will be needed. We conclude, however, that a more complete evaluation of the effectiveness of an antihypertensive regimen should include its effects on vascular smooth muscle in addition to effects on blood pressure.

**Acknowledgments**

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The C$_{19}$-Mineralocorticoids in Hypertension

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SUMMARY The excretion rates of the C$_{19}$-mineralocorticoids, 16$_{b}$-hydroxy-DHEA and 16-oxo-androstenediol, were measured in subjects with low-renin essential hypertension and toxemia of pregnancy. C$_{19}$-mineralocorticoid excretion in low-renin essential hypertension ranged from 40-760 ng per day. No significant difference in 16$_{b}$-hydroxy-DHEA and 16-oxo-androstenediol excretion was found between these subjects and normal controls. Subjects with toxemia of pregnancy excreted between 350 and 2500 ng per day of these steroids. There was no significant difference between toxemic and normal pregnancy. Thus, 16$_{b}$-hydroxy-DHEA and 16-oxo-androstenediol probably do not play an important role in either low-renin essential hypertension or toxemia of pregnancy.

DURING previous studies of urinary mineralocorticoid activity in subjects with low-renin essential hypertension, we isolated and identified two new mineralocorticoids, 16$_{b}$-hydroxy-DHEA and 16-oxo-androstenediol. These steroids had an antinatriuretic and kaliuretic action which was equal to DOCA, and which was blocked by spironolactone, a mineralocorticoid antagonist. Because the excretion of these steroids appeared to be elevated in certain subjects with low-renin essential hypertension, we speculated that the excessive production of 16$_{b}$-hydroxy-

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