The Effect of Norepinephrine on Aortic 42K 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 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 42K turnover (rate constant for DOC = 0.0164 ± 0.0009 vs. 0.0090 ± 0.0002 min⁻¹ in controls). The responses of 42K turnover to low doses of norepinephrine (NE) were increased in DOC with an ED50 of 3.5 ± 0.8 × 10⁻⁹ vs. 2.7 ± 0.5 × 10⁻⁸ 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 42K turnover and response to NE were similar to those of the DOC group. The Anti-Hy group exhibited only small increases in 42K turnover and aortic dimensions when compared to controls. It is concluded that DOC hypertension is associated with increased response of 42K 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 42K turnover and response to NE associated with DOC hypertension.

INCREASED turnover of ions in vascular smooth muscle¹⁻³ has been associated with hypertension induced by deoxycorticosterone (DOC) + salt treatment of rats. An increased contractile response to low doses of catecholamines by isolated arteries⁴⁻⁵ 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 42K turnover in a dose-dependent manner.⁶ 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 42K 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 42K 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 42K 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 Finch¹⁴ 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.¹⁵ 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 with medium containing 0.025 mM ethylenediaminetetraacetate (EDTA).

**ISOTOPE 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 washout period just before exposure to NE. The maximal response, Δkₘₐₓ, was taken as the response to a supramaximal dose of 6 x 10⁻⁶ M. The individual responses, Δk, were normalized in terms of Δkₘₐₓ 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 remaining after 1-minute washout and the specific activity of the ⁴²K solution. This yielded results equivalent to the extrapolation of the slowly exchanging component to zero time because the extracellular constituents were cleared of ⁴²K much faster than the cells. The specific activity was derived from the ratio of μmoles K⁺ (determined by flame photometry) to counts per second (gamma well counter) in a weighed sample of ⁴²K solution. The slow ⁴²K pool (⁴²Kₘₐₓ) representing intracellular K⁺ was calculated as millimoles per kilogram, wet weight, of aorta.

**DIMENSIONS**

The tissues analyzed were incubated in the dissection solution for 1 1/2 to 3 hours. The length of the thoracic aorta was measured before the isotope incubation. The aortas were lightly blotted afterward and placed in a plastic tube for weighing. Weight-length ratio was calculated and represented as milligrams per centimeter. The wall thickness of a ring of descending aorta was determined by means of a dissection microscope and ocular micrometer. Measurements were made at three different spots and averaged. The ring was then cut and the resulting radial length taken as the circumference. Recordings of isometric tension indicated that a nitrite-sensitive contraction of about 10% maximal tension was present in both controls and treated groups at 3 hours when the thickness and circumference were measured. Although not controlled, the spontaneous contractile activity is not expected to have greatly biased differences between control and treated groups but may have altered the absolute values.

**BLOOD PRESSURE**

Systolic blood pressure was determined the afternoon before the experiment by a tail cuff technique. The rats 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.

**STATISTICS**

The mean ± standard error of the mean (SEM) are presented. Significance was determined using Student’s t-test.

**Results**

**EFFECTS OF NOREPINEPHRINE**

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 10⁻⁶ M that was 4 times greater than that of controls. The maximum change, Δkₘₐₓ, induced by 6 x 10⁻⁶ M NE was slightly higher for controls than DOC (Fig. 1, Table 1). The recovery from NE followed a similar time course in both groups. The dose-response relation for DOC was shifted to the left with an ED₅₀ about 7 times lower (P < 0.001) than that of the control group (Fig. 2). The shift in the dose-response curve was not parallel. The controls exhibited a greater slope for high doses.
ANTIHYPERTENSIVE TREATMENT

Both treatment with 6-OH-DA and the antihypertensive 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 group but not in controls. The ED_50 for NE in both control and DOC rats. In sharp contrast, the 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.

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 pressure (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 Δk_{max} (min⁻¹)</th>
<th>NE ED_{50} (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>135</td>
<td>319</td>
<td>33.6</td>
<td>9.7</td>
<td>142</td>
<td>5.5</td>
<td>32.8</td>
<td>0.0090</td>
<td>0.0151</td>
</tr>
<tr>
<td></td>
<td>±4</td>
<td>±10</td>
<td>±1.4</td>
<td>±0.2</td>
<td>±4</td>
<td>±1.4</td>
<td>±0.0002</td>
<td>±0.0004</td>
<td>±0.0008</td>
<td>±0.05</td>
</tr>
<tr>
<td>DOC</td>
<td>7</td>
<td>225</td>
<td>300</td>
<td>47.1*</td>
<td>13.0*</td>
<td>190*</td>
<td>5.7</td>
<td>44.9*</td>
<td>0.0164*</td>
<td>0.0125</td>
</tr>
<tr>
<td></td>
<td>±6</td>
<td>±7</td>
<td>±1.8</td>
<td>±0.6</td>
<td>±3</td>
<td>±0.2</td>
<td>±1.7</td>
<td>±0.0009</td>
<td>±0.0006</td>
<td>±0.08</td>
</tr>
<tr>
<td>Control + Anti-Hy</td>
<td>8</td>
<td>108</td>
<td>315</td>
<td>31.3</td>
<td>9.1</td>
<td>140</td>
<td>6.0</td>
<td>31.7</td>
<td>0.0092</td>
<td>0.0191</td>
</tr>
<tr>
<td>Hy</td>
<td>±3</td>
<td>±20</td>
<td>±1.3</td>
<td>±0.3</td>
<td>±8</td>
<td>±0.1</td>
<td>±1.1</td>
<td>±0.0003</td>
<td>±0.0018</td>
<td>±0.7</td>
</tr>
<tr>
<td>DOC + Anti-Hy</td>
<td>10</td>
<td>146*</td>
<td>338</td>
<td>39.6*</td>
<td>11.0*</td>
<td>164*</td>
<td>6.0</td>
<td>40.6*</td>
<td>0.0117*</td>
<td>0.0141</td>
</tr>
<tr>
<td></td>
<td>±3</td>
<td>±8</td>
<td>±1.4</td>
<td>±0.3</td>
<td>±3</td>
<td>±0.1</td>
<td>±2.0</td>
<td>±0.0099</td>
<td>±0.0010</td>
<td>±0.8</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.
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 both an increased 42K and 36Cl turnover and increased effect of NE on 42K turnover, however, provides an ionic basis for increased contractile activity. It has been suggested that increased permeability of the membrane to Na+, K+, and Cl− would be associated with partial membrane depolarization, which in turn is an important signal for excitation-contraction coupling. The relatively high Cl− content of vascular smooth muscle would be an important factor in this process. The chloride equilibrium potential, ECl, is estimated to be 20 mV more electronegative than the membrane potential. Increased membrane permeability to Cl− therefore would be expected to depolarize the membrane and both counteract the effects of increased K+ permeability and augment the effects of increased Na+ permeability. Decreased electrogenic transport of Na+ is another ionic process which might lead to depolarization. It has been proposed that this occurs in vascular smooth muscle during perinephritic hypertension in the dog, but the finding needs to be confirmed in other models.

NE has been shown to depolarize vascular smooth muscle in a dose-dependent manner, very likely by increasing membrane permeability to ions. Therefore it seems reasonable to speculate that a shift in the dose-response relation between NE and 42K turnover may be closely related to a shift in membrane depolarization and subsequent contraction. Such shifts would have the effect of increasing 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 6-OH-DA 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

We thank Janet Schnauss Cohen for able assistance.
The C$_{19}$-Mineralocorticoids in Hypertension

JORDAN A. SENNETT, LARRY R. YARBRO, PAUL E. SLATON, JOHN W. HOLLIFIELD, AND GRANT W. LIDDLE

SUMMARY The excretion rates of the C$_{19}$-mineralocorticoids, 16$\beta$-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 $\mu$g per day. No significant difference in 16$\beta$-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 $\mu$g per day of these steroids. There was no significant difference between toxemic and normal pregnancy. Thus, 16$\beta$-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$\beta$-hydroxy-DHEA and 16-oxo-androstenediol. These steroids had an antinatriuretic and kaliuretic action which was equal to DOC, 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$\beta$-hydroxy-DHEA and 16-oxo-androstenediol was a cause of low-renin essential hypertension. However, further studies showed that the production of 16$\beta$-hydroxy-DHEA and 16-oxo-androstenediol was ACTH dependent. When we found that the blood pressure of the majority of low-renin subjects remained elevated despite the suppression of 16$\beta$-hydroxy-DHEA and 16-oxo-androstenediol excretion with glucocorticoid treatment, we concluded that these C$_{19}$-mineralocorticoids probably did not play an important role in the etiology of low-renin essential hypertension.

Nevertheless, we have continued to investigate these new mineralocorticoids. In particular, we have tested a large number of C$_{19}$-steroids for mineralocorticoid activity and structure. Furthermore, we have measured C$_{19}$-mineralocorticoid excretion in a series of subjects with low-renin essential hypertension and with 

From the Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee.

These studies were supported in part by the following grants-in-aid from the National Institutes of Health, U.S.P.H.S.: F 32 HL 05146 and 2P50 HL 14192; 2 MOH-RR-95; and Howard Hughes Medical Institute.

Received June 14, 1976; accepted for publication December 30, 1976.
The effect of norepinephrine on aortic 42K turnover during deoxycorticosterone acetate hypertension and antihypertensive therapy in the rat.
A W Jones, P D Sander and D L Kampschmidt

Circ Res. 1977;41:256-260
doi: 10.1161/01.RES.41.2.256

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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