Aldosterone Binding Sites in Aortic Cell Cultures From Spontaneously Hypertensive Rats
NANCY R. NICHOLS, PH.D., CHARLES E. HALL, PH.D., AND WALTER J. MEYER, III, M.D.

SUMMARY Spontaneously hypertensive rats and rats made hypertensive by deoxycorticosterone-salt treatment have in common increased Na⁺ and K⁺ permeability and transport in their aortic cells. These changes may be important factors in the development of the hypertensive state and may be mediated by mineralocorticoid binding to intracellular sites in the aorta. Therefore, we examined ³H-alderosterone binding in aortic cell cultures from spontaneously hypertensive rats and normotensive Wistar-Kyoto rats. Vascular corticoid binding sites in the two strains were compared by Scatchard analysis of Kₐ and Bₚmax, pH and temperature stability, and subcellular binding. By all of these criteria we found that aldosterone binding sites in cultured aortic cells are similar in the hypertensive and normotensive rats. These results indicate that the underlying genetic defect in spontaneous hypertension is not an intrinsic cellular defect which alters mineralocorticoid binding in the aorta.

(Hypertension 4: 646-651, 1982)

KEY WORDS • aorta • cell culture • dexamethasone • hypertension • mineralocorticoid • vascular smooth muscle • Wistar-Kyoto rat

THE multiplicity of adrenal steroid effects on blood pressure regulation and the development of hypertension is becoming apparent. Initially, expansion of the blood volume and extracellular fluid volume, secondary to excessive salt and water retention by the kidney, was identified as a major cause of mineralocorticoid-induced hypertension. Physiological evidence derived from rat hypertension models suggests that mineralocorticoids also have a direct effect on the blood vessel wall during the prehypertensive and hypertensive states. These effects include vessel wall hypertrophy¹ and significant increases in cellular ion content and ion turnover.² ³ Similar structural and functional changes have been found in various arteries of spontaneously hypertensive rats but not in normotensive control rats.⁴ ⁵ ⁶

Recently, we have presented evidence for displaceable mineralocorticoid and glucocorticoid binding in smooth muscle cells and fibroblasts cultured from rat aorta.⁷ The data support the hypothesis that vascular smooth muscle may be a target tissue for corticoid hormones. Vascular steroid binding sites differ from classical mineralocorticoid and glucocorticoid receptors, however, and may be involved in physiological effects other than classical mineralocorticoid and glucocorticoid effects. For example, a receptor mechanism may exist for steroid actions in vascular cells that can contribute to the overall development of high blood pressure.

Previously, the aldosterone binding properties of classical renal mineralocorticoid receptors were shown to be equivalent in spontaneously hypertensive rats (SHR) and genetically related Wistar-Kyoto (WKY) normotensive control rats.⁸ In the present investigation we have compared aldosterone binding characteristics of aortic cells cultured from SHR and WKY rats.

Materials and Methods

Chemicals

The chemicals ¹H-Aldosterone (¹H-A, 57-82 Ci/mole) and ¹H-dexamethasone (¹H-DM, 36-42 Ci/mole) were purchased from New England Nuclear (Boston, Massachusetts). Nonradioactive aldosterone and dexamethasone were obtained from Sigma (St Louis, Missouri). Steroids were stored in absolute eth-
an ol at 4°C and were checked for purity by thin layer chromatography. Tissue culture media components, Hank’s balanced salt solution and trypsin were purchased from Gibco (Grand Island, New York); calf thymus DNA and human gamma globulin from Sigma (St Louis, Missouri); and Sephadex G-25 from Pharmacia (Piscataway, New Jersey).

Animals

SHR and WKY rats from Charles River Laboratories (Wilmington, Massachusetts) were matched for age (12 to 14 weeks) and sex (male) and were maintained on Ralston Purina Lab Chow and deionized water. Systolic blood pressures were measured in conscious rats by the tail-cuff method with a programmed electrophysymomanometer and physiograph (Narco Biosystems, Houston, Texas). Rats with values above 150 mm Hg were regarded as hypertensive.

Aortic Cell Cultures

Animals were exsanguinated by cardiac puncture under ether anesthesia and sections of the thoracic aorta were removed under sterile conditions to dishes containing minimal essential medium (MEM) with Earle’s salts and supplemented with nonessential amino acids, 27 mM sodium bicarbonate, 15% fetal calf serum (FCS), penicillin (25 units/ml), streptomycin (25 µg/ml), and amphotericin B (2.5 µg/ml). The explants were scraped with a scalpel to remove the adventitia, washed with fresh MEM, and then finely minced in 60 mm plastic tissue culture dishes for culturing in MEM at 37°C under a humidified atmosphere of 10% carbon dioxide, 90% air. Aortic cell lines were serially subcultured in 1 ml of 0.02 M Tris buffer (pH 7.5) containing 0.5 M KCl, 1.5 mM EDTA and 20% (v/v) glycerol (column buffer), and sonicated to break up the cell and nuclear membranes. After centrifugation, the supernatant was assayed for DNA concentration and bound radioactivity determined by the gel filtration method described for whole cells. The crude cytoplasmic fraction (800 X g supernatant) was centrifuged at 1600 × g for 20 minutes, the supernatant was assayed for DNA concentration and bound radioactivity determined by the method described for whole cells. The residual pellet contained less than 5% of the nuclear binding. The crude cytoplasmic fraction (800 X g supernatant) was centrifuged at 1600 × g for 20 minutes, then assayed for bound radioactivity. The following sources of error in the aldosterone binding experiments were included: counting error, error in receptor determination, and error due to receptor in-
stability. The coefficients of variation were 2–10% for the sample counts and 15% for the background counts. The DNA intra-assay variation was 5–10%. Additional errors in the binding assay were due to receptor instability.7,13 These errors were introduced when assay time, assay temperature or sonication time was varied.

**Results**

**Blood Pressure Measurements**

All SHR rats had blood pressures in excess of 150 mm Hg. The highest blood pressure recorded for a normotensive WKY rat was 142.5 mm Hg. The SHR strain had a significantly \( p < 0.001 \) elevated systolic blood pressure compared to the normotensive strain: 175 ± 11.5 mm Hg \( (n = 8) \) and 132 ± 6.8 mm Hg \( (n = 7) \) respectively.

**Scatchard Analysis of \(^3\text{H}-\text{Aldosterone Binding}\)**

A complete Scatchard plot\(^a\) of \(^3\text{H}-\text{A}\) binding in aortic cells cultured from WKY rats is shown in top of figure 1. The curvilinear plot in the top of figure 1 was resolved into two lines by a graphical method\(^7\) shown in the bottom section of figure 1: line \( a \) represents aldosterone binding to higher affinity sites, previously\(^7\) termed corticoid receptor I \( (K_d = 0.44 \text{ nM}, B_{\text{max}} = 56.1 \text{ moles} \times 10^{-18} / \mu\text{g DNA}) \), and line \( b \) represents aldosterone binding to lower affinity sites (corticoid receptor II; \( K_d = 6.08 \text{ nM}, B_{\text{max}} = 316.5 \text{ moles} \times 10^{-18} / \mu\text{g DNA}) \).

The variation in the binding data within the animal population is depicted geometrically in figure 1 left. The variation of binding at 0.125 nM \(^3\text{H}-\text{A}\), a point in the corticoid I portion of the curve, was less than the variation in binding at 5 nM \(^3\text{H}-\text{A}\), a point in the corticoid receptor II portion of the curve: 15.64 ± 1.81 and 147.70 ± 51.24 moles \( \times 10^{-18} / \mu\text{g DNA} \) respectively. The coefficients of variation were 11.6% for corticoid receptor I binding and 34.7% for corticoid receptor II binding. The corticoid receptor II sites are not saturated by aldosterone under the experimental conditions;\(^7\) this may account for the greater variation in the data.

Scatchard plot analysis of aldosterone binding in aortic cell cultures from all WKY and SHR rats is summarized in table 1. Both strains exhibited similar dissociation constants and binding capacities for corticoid receptors I and II. The large variation in \( B_{\text{max}} \) for corticoid receptor I in the SHR strain was due to a single rat cell line which exhibited 6–7 times the binding of the other seven cell lines. If this animal is left out of the analysis, then the mineralocorticoid binding capacity is 69.0 ± 27.8 moles \( \times 10^{-18} / \mu\text{g DNA} \).

**Blood Pressure and Binding Capacity**

Systolic blood pressure and the aldosterone binding capacity of both aortic corticoid receptors are poorly correlated. Blood pressure accounts for only 10% \( (r^2) \) of the variance observed in \( B_{\text{max}} \) of corticoid receptors I and II in SHR aortic cells. In aortic cells of nonhypertensive rats, blood pressure accounts for 35% and 25% of the variance observed in \( B_{\text{max}} \) of corticoid receptor I and corticoid receptor II respectively.

---

**Figure 1.** A composite Scatchard plot of \(^3\text{H}-\text{aldosterone binding in aortic cells cultured from five different Wistar-Kyoto rats (WKY).** Left: Curvilinear Scatchard plot of the binding data. The mean ± standard deviations \( (sd) \) of the X and Y coordinates are denoted by the points and elliptic fields, respectively. Right: Graphic resolution of the composite curve in top figure. Line \( a \) represents the high affinity, low capacity binding sites (corticoid receptor I). Line \( b \) represents the low affinity, high capacity binding sites (corticoid receptor II).
Scatchard Analysis of 3H-Dexamethasone Binding

Dexamethasone binds to a set of displaceable sites with the same affinity as aldosterone for corticoid receptor II sites, but with higher capacity. Therefore, binding of the potent glucocorticoid, dexamethasone, was examined in aortic cells from WKY and SHR rats. A representative saturation curve and Scatchard plot are shown in figure 2. Saturation was achieved at 10-15 nM dexamethasone (fig. 2 left). Unlike the plot of aldosterone binding in the aorta, the Scatchard plot of dexamethasone binding is linear (fig. 2 right). The results of Scatchard analysis of three WKY and three SHR experiments were compared: $K_d = 7.99 \pm 2.54$ nM and $B_{max} = 10,230 \pm 1,700$ moles $\times 10^{-17}/\mu g$ DNA and $10,370 \pm 4,800$ moles $\times 10^{-18}/\mu g$ DNA respectively. The differences in the binding statistics were not significant.

Subcellular Binding

The subcellular aldosterone binding in WKY and SHR rats is compared in table 2. The experiments were performed on pairs of WKY and SHR aortic cell cultures incubated at 37°C for 30 minutes with 1 nM 3H-A with and without 1500-fold excess of nonradioactive aldosterone. At 1 nM 3H-A the corticoid receptor I sites were saturated, however, the ratio of occupied corticoid receptor I sites to occupied corticoid receptor II sites is approximately one. Therefore, it is not known if the binding recovered in the nuclear fraction represents steroid bound to one or both sites. Since binding was equivalent in 100,000 x g supernatants, the latter was used to estimate cytosol binding. The fractiona-

### Table 1. Scatchard Analysis of Aldosterone Binding in WKY and SHR Rats (± so)

<table>
<thead>
<tr>
<th>Strain (N)</th>
<th>Corticoid receptor I</th>
<th>Corticoid receptor II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_d$ (nM)</td>
<td>$B_{max}$ (moles $\times 10^{-18}/\mu g$ DNA)</td>
</tr>
<tr>
<td>WKY (7)</td>
<td>0.49 ± 0.20</td>
<td>77.2 ± 40.9</td>
</tr>
<tr>
<td>SHR (8)</td>
<td>0.47 ± 0.25*</td>
<td>115.3 ± 133.7*</td>
</tr>
</tbody>
</table>

*Not significant at the 5% level.

### Table 2. Percentage of Aldosterone Binding in Subcellular Fractions of WKY and SHR Rats (± so)

<table>
<thead>
<tr>
<th>Strain</th>
<th>No.</th>
<th>19,000 x g supernatant</th>
<th>Nuclear pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>3</td>
<td>74.6 ± 7.4</td>
<td>25.4 ± 7.4</td>
</tr>
<tr>
<td>SHR</td>
<td>3</td>
<td>70.1 ± 10.3*</td>
<td>29.9 ± 10.3*</td>
</tr>
</tbody>
</table>

*Not significant at the 5% level.

**Figure 2.** A typical saturation curve and Scatchard plot of 3H-dexamethasone binding. Left: The saturation curve of aortic cell cultures incubated with varying concentrations of 3H-dexamethasone for 30 minutes at 37°C. Cells were disrupted by sonication and assayed for bound radioactivity. Data are corrected for non-specific binding. Right: Scatchard plot for curve in top figure. The line was determined by linear regression analysis of the data ($r = -.99$).
tion procedure did not decrease the recovery of specifically-bound radioactivity since the nuclear binding plus the 19,000 × g supernatant binding closely approximated (101%) the whole cell binding; the sum of the binding in the two fractions was used as the denominator in table 2. Binding in the residual pellet was not included in the estimate of nuclear binding. The subcellular binding was similar for both strains with 70–75% of the binding sites recovered in the 19,000 × g supernatant and 25% to 30% recovered in the nuclear pellet. The percentage of binding in the subcellular fractions was unaffected by the number of whole cell binding sites which varied from experiment to experiment.

pH and Temperature Stability

Cells cultured from WKY and SHR rats were incubated with 1 nM 3H-A at 37°C for 30 minutes and the pH and temperature stability of the complexes compared for the two strains. Preliminary experiments indicated that nonspecific binding did not change with buffer of varying pH. Therefore, only total binding was determined in subsequent pH stability studies. The data in figure 3 demonstrate that the total binding for both strains was very stable from pH 5 to pH 10, but that some loss of binding did occur at pH 11. The results at each pH were compared and all differences between the two strains were not statistically significant.

In contrast to their marked pH stability, aortic aldosterone binding sites are extremely thermolabile. When preformed complexes were incubated at various temperatures for 15 minutes and then assayed for bound radioactivity, a sharp decline in activity was apparent from 21°C to 37°C for both strains (not shown). Temperature stability experiments performed at 25°C for WKY and SHR rats showed similar time- and temperature-dependent dissociation of bound aldosterone (fig. 4). Statistical analysis of the data at each time point showed no significant difference between the two strains. The half-life of aortic aldosterone binding is 30–35 minutes at 25°C.

Discussion

In spite of highly significant differences in systolic blood pressure between the two strains, aldosterone binding sites in SHR and WKY aortic cells were similar in terms of affinity, capacity, subcellular binding, and pH- and temperature-stability. Differences in aldosterone binding statistics between the strains were not obscured by variation in the binding data within an animal population (fig. 1 left), since a similar pattern of variation was observed for seven repetitive experiments on the same subculture of a rat aortic cell line. The steroid specificity of aldosterone binding was not compared; these studies are difficult to interpret in the aortic cell system because of the relative lack of specificity between hormones with classical glucocorticoid and mineralocorticoid effects in competing for corticoid I or corticoid II sites. The affinity and capacity of dexamethasone binding sites were also indistinguishable in the genetically hypertensive rats and their normotensive controls. In fact, aldosterone and dexamethasone binding statistics were the same as those
previously reported for Sprague-Dawley rats, which were neither matched for age nor monitored for blood pressure. The cell morphology and growth pattern of SHR and WKY aortic cell lines were indistinguishable and were similar to those already described for aortic smooth muscle cell cultures selected from SHR and WKY rats by Sands et al. The proportion of smooth muscle cells and fibroblasts in the aortic cultures did not contribute to error in the binding data since the $K_d$ and $B_{max}$ of predominantly smooth muscle cell cultures were not significantly different from predominantly fibroblast cultures.

A comparison of mineralocorticoid binding in the aorta of SHR and WKY rats was of particular interest because similar ionic disturbances have been found in aortic smooth muscle of SHR and DOCA-salt treated rats. Mineralocorticoid involvement is also suggested by the fact that adrenalectomy delays the development of hypertension in SHR rats. Several studies indicate that mineralocorticoids and glucocorticoids increase vascular reactivity to catecholamines and hyperreactivity of the sympathetic nervous system has been implicated in the pathophysiology of hypertension in the SHR strain.

Feldman examined SHR rats for defects in binding to classical renal mineralocorticoid receptors. Mineralocorticoid receptors in kidney slices of SHR and WKY rats exhibited similar properties of affinity, capacity, specificity, and nuclear uptake. These studies on aortic aldosterone binding differ from the renal experiments in two ways. First, on the basis of steroid specificity, aortic aldosterone binding sites differ from classical mineralocorticoid and glucocorticoid receptors. Aortic binding sites may represent a new class of steroid receptors with physiological effects different from classical renal receptors. Second, aortic cells were in culture for 8–20 passages. Therefore, the results exclude intrinsic genetic differences in aortic cell aldosterone binding, but do not exclude in vivo differences due to extrinsic factors present in hypertensive and normotensive rats.

References

Aldosterone binding sites in aortic cell cultures from spontaneously hypertensive rats.
N R Nichols, C E Hall and W J Meyer, 3rd

Hypertension. 1982;4:646-651
doi: 10.1161/01.HYP.4.5.646

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/4/5/646

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/