Inhibition of Nuclear Polyploidy by Propranolol in Aortic Smooth Muscle Cells of Hypertensive Rats

MARK LEITSCHUH AND ARAM V. CHOBANIAN

SUMMARY The ability of propranolol to inhibit the development of polyploidy in aortic vascular smooth muscle cells associated with hypertension was studied in deoxycorticosterone (DOC)-salt treated rats. Six-week treatment with DOC-salt resulted in significant increases in systolic blood pressure, heart weight, and aortic weight in treated animals compared to increases in uninephrectomized controls. Additionally, the percentage of tetraploid nuclei in aortic smooth muscle cells increased to 17.0 ± 0.2% in DOC-salt treated rats versus 7.8 ± 0.3% in normotensive controls. Administration of propranolol (500 mg/L in drinking water) did not inhibit the development of hypertension for up to 4 weeks or the associated increase in cardiac or aortic weight in DOC-salt-treated rats, but did prevent the increase in polyploidy of aortic smooth muscle cell nuclei (8.9 ± 0.9% in propranolol-treated rats compared to 7.8 ± 0.3% in normotensive controls). These results indicate that propranolol inhibits the development of hypertension-induced polyploidy in aortic smooth muscle cells of DOC-salt-treated rats and that factors other than blood pressure may be important in this change.

(Hypertension 9 [Suppl III]: IIM06-III-109, 1987)

KEY WORDS • smooth muscle cell polyploidy • hypertension • propranolol

INCREASED aortic mass associated with hypertension may make a major contribution to cardiovascular pathology. Although many factors are important to this change, much of the increased aortic mass can be attributed to changes in smooth muscle cells (SMCs) located in the media of the artery. Such changes include cellular hypertrophy, hyperplasia, and the more recently described phenomenon of hyperploidy. Cellular hypertrophy is the process by which the individual cells increase in size, whereas hyperplasia involves increases in cell number secondary to normal diploid replication. Hyperpliosis is the increase in DNA content within an individual cell without increases in cell number. The normal cell nucleus has two copies of each chromosome and is termed diploid. In the process of hyperploidy, DNA duplication occurs in the cell without subsequent karyokinesis or cytokinesis, thus resulting in polyploidy. Little is known about the significance of hyperploidy, but given the large increase in the DNA content of these cells, changes in metabolism and reactivity to stimuli would not be unexpected.

Because of these potentially important considerations, we have been studying possible etiological factors contributing to the development of SMC hyperploidy in rats with deoxycorticosterone (DOC)-salt hypertension, a model that has been well characterized in our laboratory. In this model, there is evidence for increased sympathetic discharge in the heart and blood vessels. Other studies in the rat and rabbit have suggested that sympathetic stimulation may have an important trophic effect on the vasculature. We therefore designed a study in which the β-adrenergic nervous system would be modified in hypertensive animals by treatment with the β-blocking drug propranolol, with the aim of preventing the development of hyperploidy in aortic SMCs. Because of the minimal blood pressure-lowering effects of propranolol in the DOC-salt model of hypertension, we also hoped to disassociate the blood pressure effects of the treatment from other possible mechanisms contributing to hyperploidy.

Materials and Methods

Male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA, USA) weighing between 175 and 200 g underwent uninephrectomy and, following 1 week of recovery, were divided into three groups.
Group 1 consisted of normotensive controls given no treatment. Group 2 consisted of rats with DOC-salt hypertension, induced with deoxycorticosterone pivalate (15 mg/kg s.c., biweekly) and 1% saline as drinking water. Group 3 consisted of rats with DOC-salt hypertension (induced as for Group 2) that were given propranolol, 500 mg/L, in the 1% saline drinking water. The animals were caged individually. The average dose of propranolol, which was determined by measuring the volume of drinking water biweekly, was 80.6 ± 2.5 mg/kg/day. Systolic blood pressure was measured using the tail cuff method at the initiation of the study and at 2-week intervals thereafter. At 6 weeks, the animals were killed with a lethal dose of sodium pentobarbital and body, heart, and aortic weights were measured. Aortic SMCs were isolated and nuclear ploidy determined as described below.

Isolation of Aortic Smooth Muscle Cells

Aortic SMCs were isolated by enzymatic dispersion techniques described previously. The aorta was removed from the aortic arch to the bifurcation. The media was dissected free of the adventitia and placed in modified Hanks's salt solution containing 0.2 mM CaCl₂, pH 7.4. The tissue was minced with a Brinkman Tissue Chopper (Mickle Laboratory Engineering, Surry, UK) and digested in 5 ml of enzyme mixture containing 750 U collagenase (Type 1, Sigma), 20 U elastase (Type III, Sigma), 3 mg lima bean trypsin inhibitor (Sigma), 100 U DNase (Type 1, Worthington), and 1% bovine serum albumin (Fraction V, Sigma). After 75 to 90 minutes, the reaction was terminated by the addition of 20 ml of Dulbecco's modified Eagle's medium containing 10% fetal calf serum. The suspension was dispersed by aspiration 10 times through a 13-gauge needle and then by filtration through a 105-μm mesh. Undigested tissue on the mesh was redigested in 5 ml of enzyme mixture for 45 minutes and again filtered. The combined filtrate was centrifuged at 100 g for 10 minutes to obtain isolated cells.

Determination of Ploidy of Aortic Smooth Muscle Cell Nuclei

Freshly isolated cells were pelleted and resuspended in 500 μl of 1% Triton X-100 to obtain nuclei and stained with 50 μl propidium iodide (50 μg/ml). The preparation was filtered through a 44-μm mesh and the fluorescence determined using a FACS system (Becton-Dickinson, Sunnyvale, CA, USA) interfaced with a PDP 11/23 computer (Digital Equipment, Maynard, MA, USA). Graphic display of the distribution of the diploid and tetraploid nuclei was obtained and the percentage of tetraploid nuclei determined.

Statistical Analysis

Student's t test was used to compare all groups except for the 6-week blood pressures, in which case the Wilcoxon rank sum test was used due to large variability.

Results

Systolic Blood Pressure

The systolic blood pressure response to treatment in the three groups is summarized in Table 1. At 4 weeks, there was a significant rise in blood pressure in the DOC-salt hypertensive group compared to the control group (167 ± 8 vs 122 ± 3 mm Hg), but there was no statistical difference in blood pressure between the animals treated with DOC-salt alone and those treated with DOC-salt and propranolol (167 ± 8 vs 160 ± 14 mm Hg). At 6 weeks, the blood pressures of both the DOC-salt group and the DOC-salt plus propranolol group remained elevated compared to pressures in the control group, with pressures in the propranolol group somewhat lower than pressures in the DOC-salt hypertensive group (188 ± 6.0 vs 154.1 ± 10.0 mm Hg).

Heart Weight and Aortic Weight

The mean aortic weight was 0.113 ± 0.009 g for control rats, 0.131 ± 0.008 g for the DOC-salt hypertensive rats, and 0.111 ± 0.005 g for the DOC-salt plus propranolol rats. However, when expressed as a percentage of body weight, there was a statistically significant increase above controls in aortic weight as well as in heart weight in both the DOC-salt hypertensive group and the DOC-salt hypertensive group treated with propranolol (Table 2). These findings suggest that propranolol did not prevent the increase in aortic or cardiac mass associated with DOC-salt hypertension.

Table 1. The Effect of Propranolol on Systolic Blood Pressure in DOC-Salt and DOC-Salt Plus Propranolol-Treated Rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Baseline</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive control rats</td>
<td>102 ± 5</td>
<td>106 ± 5</td>
<td>122 ± 4</td>
<td>126 ± 4</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>DOC-salt hypertensive rats</td>
<td>99 ± 3</td>
<td>131 ± 6</td>
<td>167 ± 9</td>
<td>188 ± 6</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 4)</td>
</tr>
<tr>
<td>DOC-salt hypertensive rats</td>
<td>118 ± 4</td>
<td>139 ± 5</td>
<td>160 ± 7</td>
<td>154 ± 10</td>
</tr>
<tr>
<td>given propranolol</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
</tbody>
</table>

Values represent means ± SE.

DOC-salt hypertension was induced by biweekly s.c. injection of deoxycorticosterone pivalate and 1% saline as drinking water. Propranolol was given in drinking water; average dose, 80.6 ± 2.5 mg/kg/day.

*p < 0.01, **p < 0.001, ***p < 0.05, compared with normotensive controls (by Student's t test).
III-108 1986 BLOOD PRESSURE COUNCIL SUPPL III HYPERTENSION, VOL 9, NO 6, JUNE 1987

TABLE 2. The Effects of Propranolol on Heart and Aortic Weight in DOC-Salt Hypertension

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Heart weight (% body wt)</th>
<th>Aortic weight (% body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive control rats</td>
<td>0.24 ± 0.006</td>
<td>0.024 ± 0.001</td>
</tr>
<tr>
<td>DOC-salt hypertensive rats</td>
<td>0.39 ± 0.002*</td>
<td>0.039 ± 0.002*</td>
</tr>
<tr>
<td>DOC-salt hypertensive rats given propranolol</td>
<td>0.37 ± 0.002*</td>
<td>0.036 ± 0.002*</td>
</tr>
</tbody>
</table>

Values represent means ± SE. See Table 1 for dosages.
*p < 0.001 compared with normotensive controls. Differences between DOC-salt and DOC-salt plus propranolol groups not significant above p values obtained by Student’s t test.

Nuclear Ploidy of Aortic Smooth Muscle Cells

Table 3 summarizes the results of the ploidy determinations of the three groups. As expected, there was an increase in the percentage of tetraploid nuclei in the DOC-salt hypertensive group to 17.0 ± 0.2%. However, propranolol prevented the development of polyploidy in the face of DOC-salt hypertension such that there was no statistically significant difference between the percentage of tetraploid nuclei in the DOC-salt hypertensive group treated with propranolol and the normotensive controls (8.9 ± 0.9 vs 7.8 ± 0.3%).

Discussion

The purpose of this study was to examine the possible role of the β-adrenergic nervous system in the development of hyperploidy associated with DOC-salt hypertension in the rat. The most significant finding is that the β-adrenergic blocking drug propranolol inhibits the development of hyperploidy in DOC-salt hypertension. Work in our laboratory has previously shown that hyperploidy develops within 4 weeks in the DOC-salt model of hypertension and that, once established, it does not regress in spite of prolonged blood pressure controlled, but that in spite of preventing blood pressure rise, captopril did not suppress the hyperplastic response. Sen also found large differences in the degree of reversal of cardiac hypertrophy brought about by various antihypertensive drugs and in various models of hypertension. Sen suggested that a combination of various factors, especially adrenergic factors, plays an important role in the modulation of myocardial structures in response to changes in blood pressure.

Although propranolol prevented the development of hyperploidy, it did not significantly influence cardiac or aortic mass. This suggests that other potential mechanisms that increase aortic mass, such as cellular hypertrophy, hyperplasia, or increased production of extracellular matrix, respond to different stimuli than the mechanisms that trigger hyperploidy. Our previous work has indicated that both total collagen and elastin of aortas of DOC-treated rats increase in proportion to the increased wet weight of aortic tissue, and that blood pressure lowering does not readily reverse these changes.

In summary, we have shown that propranolol inhibi-
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its the development of hyperploidy in aortic SMCs associated with DOC-salt hypertension in the rat. This suggests a possible role of the β-adrenergic nervous system in the development of polyploidy, but other actions of propranolol independent of its β-blocking properties could also be responsible for the effect.

References

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Hypertension. 1987;9:III106
doi: 10.1161/01.HYP.9.6_Pt_2.III106

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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