Alterations in Vascular Smooth Muscle Mass in the Spontaneously Hypertensive Rat

Role of Cellular Hypertrophy, Hyperploidy, and Hyperplasia

Gary K. Owens and Stephen M. Schwartz
From the Department of Pathology SM-30, University of Washington, Seattle, Washington

SUMMARY. In a previous brief report we demonstrated that differences in aortic smooth muscle mass between spontaneously hypertensive and Wistar-Kyoto rats were due to smooth muscle cell hypertrophy, without hyperplasia. Smooth muscle cell hypertrophy, however, was accompanied by an increase in the frequency of polyploid cells. This study reports (1) the relationship between changes in smooth muscle cell mass and DNA ploidy, (2) the proportion of the increase in mass of smooth muscle in spontaneously hypertensive rats that can be accounted for by polyploid cells, and (3) the time-course of changes in ploidy during the development of hypertension. Flow microfluorimetric and Feulgen-DNA microspectrophotometric measurements demonstrated that the frequency of polyploid smooth muscle cells was 2-3 times greater in spontaneously hypertensive rats than in Wistar-Kyoto rats at 3 months of age and older. The frequency of polyploid cells increased with age and level of blood pressure. No differences in the frequency of polyploid cells were apparent between prehypertensive 1-month spontaneously hypertensive and Wistar-Kyoto rats. By cytospectrophotometric analysis, spontaneously hypertensive rat diploid, tetraploid, and octaploid smooth muscle cells had 36%, 136%, and 377%, respectively, the protein content of Wistar-Kyoto rat diploid cells. The increase in mean cellular protein (53% by cytospectrophotometry) in spontaneously hypertensive rats could account for the total increase (56%) in aortic smooth muscle mass, measured by morphometry. Thus, smooth muscle cell hypertrophy alone can account for the increased mass of smooth muscle in spontaneously hypertensive rat aortas, while the majority of change in smooth muscle mass is due to the increased frequency and mass of polyploid cells. (Circ Res 51: 280–289, 1982)

CHRONIC hypertension in both experimental animals (Folkow et al. 1973; Pfeffer and Frohlich, 1973; Berecek and Bohr, 1977; Ferrone et al., 1979) and humans (Brod, 1963; Korner et al., 1973; Frohlich, 1977) is characterized by an increase in peripheral resistance. Folkow and others (Folkow et al., 1973; Lundgren et al., 1974; Berecek and Bohr, 1977) have shown that a major component of this increased resistance cannot be abolished by pharmacological relaxation of vascular smooth muscle. Based on this and other hemodynamic findings, they have proposed that this "nonfunctional" or so-called "structural" component of vascular resistance was due to an increase in vessel thickness and smooth muscle mass. This confers a mechanical advantage so that at any given level of smooth muscle activity, vascular resistance would be greater in hypertensives than controls.

In agreement with physiological studies, morphometric studies have shown an increase in wall thickness in both large and small arteries of hypertensive animals (Ichijma, 1969; Wolinsky, 1970; Weiner et al., 1977; Warshaw et al., 1979; Olivetti et al., 1980) and man (Furuyama, 1962), and have established that this increase was due, at least in part, to an increase in smooth muscle mass. It was not clear, however, whether the increased mass was due to an increase in cell number (hyperplasia) or an increase in cell size (hypertrophy). Based on observations of increased numbers of vascular smooth muscle cells labeling with $^{3}$H-thymidine (Crane and Dutta, 1963; Fernandez and Crane, 1970; Bevan, 1976; Rorive et al., 1980) and an increase in DNA content per unit vessel length, Bevan et al. (1976; 1980) concluded that hyperplasia was the principal mechanism. In contrast, morphometric studies by Olivetti et al. (1980) showed that the increase in smooth muscle mass following aortic stenosis could be accounted for solely by cellular hypertrophy. Likewise, based on biochemical measurements, we found no evidence for an increase in smooth muscle cell number in aortas of spontaneously hypertensive rats (SHR) relative to Wistar-Kyoto (WKY) controls, and concluded that smooth muscle cell hypertrophy alone must account for differences in smooth muscle mass (Owens et al., 1981). Furthermore, we found that smooth muscle cell hypertrophy was accompanied by an increase in the frequency of polyploid cells. However, no data were presented to demonstrate the possible relationship between cellular hypertrophy and cellular hyperploidy.

The work reported here represents an extension of our earlier study. The primary objectives were (1) to define the interrelationship between smooth muscle...
cell hypertrophy and hyperploidy (are the polyploid cells the hypertrophic cells?), (2) to determine what proportion of the total change in mass of smooth muscle in SHR can be accounted for by polyploid cells, and (3) to describe the time-course of changes in ploidy relative to changes in blood pressure.

**Methods**

**Animals**

Male rats were used throughout these experiments. Sprague-Dawley rats were obtained from Tyler Laboratories. SHR and WKY rats were obtained from Charles River Breeding Laboratories. Food and water were administered ad libitum.

**Assessment of Hypertension**

Blood pressures were measured via a tail-cuff method using a piezoelectric pressure transducer (Buffington Clinical Systems). A minimum of three readings were taken for each animal during the 2 weeks prior to sacrifice. Animals were lightly anesthetized with ether and the tail temperature was maintained with an electric heating pad. In the case of perfusion-fixed animals, pressures were also measured directly by an indwelling catheter in the left carotid artery at the time of sacrifice. In addition, heart weights were determined in the following manner: hearts were excised, perfused briefly with Hank’s buffer (pH 7.4) to remove blood, dried for 48 hours, and weighed.

**Morphometry**

Morphometric evaluation of tissue sections was used to measure differences in aortic thickness and smooth muscle mass between SHR and WKY rats. In addition, morphometric evaluations of multiple samples from the same area were used to assess smooth muscle cell hypertrophy in an intact vessel. Animals for morphometry were killed by cervical dislocation following ether anesthesia. The thoracic aorta was excised, perfused with Hank’s solution (pH 7.4), and placed in fresh Hank’s. An intimal-media preparation was made using procedures described by Wolinsky and Daly (1970). The endothelium was then scraped off with a piece of teflon. Resultant medial preparations were used for subsequent isolation of smooth muscle cells.

Smooth muscle cells were isolated as previously described by Owens et al. (1981). In brief, medial prepsations of thoracic aorta were finely minced in 0.1% collagenase (146 U/mg; Worthington) and 0.05% elastase (Type I, 32 U/mg, Sigma Chemical Company) in Hank’s buffer. This was incubated in 37°C (5% CO2, 95% air) for 3 hours. At 1-hour intervals, the enzyme mixture and tissue were gently pipetted in and out of a wide-mouth pipette to aid the tissue dissociation. Preparations were observed with an inverted phase microscope. When cells were adequately dispersed, fetal calf serum was added to a final concentration of 20% (vol/vol). Suspensions were filtered through 250-μm stainless-steel mesh to remove tissue debris and undigested tissue. The filtrate was centrifuged at 200 g for 5 minutes (as were all subsequent centrifugation steps), and the cell pellet was resuspended in Ca++-free Hank’s buffer containing 0.5 mM EGTA. Cells were allowed to equilibrate in this solution for 15-20 minutes, and then were fixed in 10% neutral-buffered formalin for subsequent microspectrophotometric measurements or for autoradiography. Cell viability was greater than 90% as measured by trypan blue exclusion. Cellular yield, determined by DNA assay, was approximately 20%, with the greatest loss occurring during the filtration step.

**Flow Microfluorimetry**

Nuclei were prepared for flow cytometry as described by Owens et al. (1981). Medial aortic preparations were incubated (37°C) for 1.5 to 2 hours in a collagenase and elastase solution as previously described. Fetal calf serum (20%) was added to the tissue centrifuged. The cell-tissue pellet was suspended in 1 ml of the fixation solution. The suspension was centrifuged, and the cells counted. The cell pellet was then resuspended in 10 ml of the suspension medium containing 0.5% Nonidet P-40 (a nonionic detergent, vol/vol), 1 mM CaCl2, 21 mM MgCl2, and 0.2% bovine serum albumin (wt/vol). Samples were vortexed gently and cooled on ice for 10-15 minutes. Suspensions were filtered through a 70 μm stainless-steel mesh to remove tissue debris. Nuclei were then syringed three times through a 26-gauge needle to ensure single nuclei. DAPI-stained nuclear isolates were observed with a Zeiss fluorescent microscope to determine the percentage of nuclear aggregates. If greater than 2%, nuclear suspensions were resyringed. Nuclear yields were about 25% as determined by DNA assay.
Measurement, acquisition, and analysis of DNA content of isolated nuclei was done on an ICP-22 flow cytometer (Ortho Diagnostic Systems Inc.), interfaced to a PDP-11/03 computer. A UGI excitation filter, a TK-400 dichroic mirror, and an LP-435 emission filter were used to quantify DAPI fluorescence. Nuclei were analyzed at a rate of less than 400 nuclei/second and DNA content histograms produced with a NS600 multichannel analyzer (Northern Scientific, Inc.). Cell cycle compartments were estimated by an adaptation of the method of Dean and Jett (1974), including fitting and subtraction of an exponential noise background. Nonlinear least squares fitting was by the method of Marquardt (1963).

Autoradiography: \(^{3}H\)-Thymidine Labeling

Animals for autoradiography were given a single intraperitoneal dose of \(^{3}H\)-thymidine (50 \(\mu\)Ci/100 g body weight; 6.7 Ci/mM; New England Nuclear) 1 hour before being killed (Schultz, 1969). Essentially, this gives an instantaneous estimate of the frequency of cells in the S phase of the cell cycle. Thymidine indices were determined by a technique developed in this laboratory, in which autoradiography is done on smooth muscle cell dispersions prepared as described above. The advantage of this technique over conventional autoradiography on sections is that large numbers of cells can be analyzed relatively rapidly. To validate this technique, conventional autoradiography (Thomas et al., 1971) on 1-\(\mu\)m sections (taken at 10-\(\mu\)m intervals in the tissue) was done in parallel with dispersion-autoradiography in initial studies. Sampling procedures were based on earlier techniques used in this laboratory, to measure the frequency of endothelial cell replication, also a rare event (Schwartz and Benditt, 1976).

Quantitative Microspectrophotometry: DNA and Protein Determinations

To verify measurements of the frequency of polyplody of smooth muscle cells in cell dispersions, we also measured ploidy by Feulgen-DNA microdensitometry. This permits actual visual observation of each cell and thus eliminates any contribution of clumping and provides information regarding the location of polyplody in the vessel wall. For Feulgen-DNA measures, aortas were fixed in 10% neutral-buffered formalin, sectioned into lengths of 10 mm, and embedded in paraffin (Humason, 1979). Sections (8 \(\mu\)m) were Feulgen-stained as described by Fand (1970). A Vickers M85 scanning-integrating microdensitometer (Vickers Instruments) was used to quantify nuclear DNA levels. All measurements were made at the Feulgen reaction-absorption maximum of 565 nm. Care was exercised to avoid cut or overlapping nuclei. At least 200 smooth muscle cell nuclei were measured from each animal. Standards of chick red blood cells (2.5 pg DNA) were processed and stained simultaneously. DNA measurements obtained by scanning cytophotometry are expressed in integrated optical density or absorbancy units (A.U.). Under the operational conditions employed, an absorbency unit was equivalent to 6.28 pg of protein. The protein content of individual cells can thus be calculated.

Statistics

All results were analyzed by Student's t-test or linear regression analysis (Snedecor and Cochran, 1967). Probabilities of 5% or less were interpreted as being statistically significant. Mean values in the text are plus or minus standard error.

Results

Assessment of Hypertension

Mean blood pressures and heart weight:body weight ratios (Table 1) of SHR were significantly greater (\(P < 0.02\)) than WKY or Sprague-Dawley rats at 3 months of age and older. Although blood pres-

![Figure 1](attachment:figure1.png)
Table 1

<table>
<thead>
<tr>
<th></th>
<th>Blood Pressure* (mm Hg)</th>
<th>Average dry heart wt (g)</th>
<th>Average body wt (g)</th>
<th>Heart wt (X10&lt;sup&gt;-3&lt;/sup&gt;)/body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-month SHR</td>
<td>150.9 ± 3.3f</td>
<td>64.5 ± 2.4</td>
<td>12.00 ± 0.45</td>
<td></td>
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<tr>
<td>1-month WKY</td>
<td>105.5 ± 3.6</td>
<td>61.3 ± 2.2</td>
<td>12.12 ± 1.15</td>
<td></td>
</tr>
<tr>
<td>3-month SHR</td>
<td>164.4 ± 2.8f</td>
<td>308.6 ± 8.9</td>
<td>8.78 ± 0.15†</td>
<td></td>
</tr>
<tr>
<td>3-month WKY</td>
<td>237.5 ± 0.018</td>
<td>291.4 ± 7.9</td>
<td>7.58 ± 0.40</td>
<td></td>
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<tr>
<td>5-month SHR</td>
<td>164.4 ± 2.8f</td>
<td>358.7 ± 5.6</td>
<td>8.88 ± 0.20†</td>
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</tr>
<tr>
<td>5-month WKY</td>
<td>284.0 ± 6.8</td>
<td>368.1 ± 6.8</td>
<td>7.73 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>1-year SHR</td>
<td>117.5 ± 3.6</td>
<td>524.0 ± 11.4</td>
<td>6.56 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>1-year WKY</td>
<td>151.8 ± 6.1f</td>
<td>430.8 ± 8.2</td>
<td>9.07 ± 0.19†</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SEM.
* Average of last three measurements made over the 2 weeks prior to sacrifice.
† Significantly greater than age-matched WKY (P < 0.02, t-test).

Morphometric Evaluations

The primary purpose of morphometric evaluations was to provide a quantitative estimate of the difference in total aortic smooth muscle mass between 5-month-old SHR and WKY rats. In addition, these analyses provided an independent means to determine whether cellular hypertrophy had occurred in SHR. As reported previously (Owens et al., 1981), the number of nuclei per 10,000 μm<sup>2</sup> of aortic media was significantly less (P < 0.02) for SHR than WKY (Table 2). Results of smooth muscle cell volume density (V<sub>smc</sub>) determinations (i.e., the fraction of total aortic medial volume occupied by smooth muscle) were not different between SHR and WKY rats, thus indicating that this decrease in nuclear density reflects cellular hypertrophy, rather than an increase in the relative amount of extracellular matrix. The decrease (P < 0.01) in nuclear volume density (V<sub>nucl</sub>) and increase (P < 0.02) in V<sub>smc</sub>:V<sub>nucl</sub> ratio in SHR likewise indicated that SHR cells were hypertrophied.

It is also apparent from Table 2 that aortic medial thickness and medial cross-sectional area were increased in SHR. Thus, total aortic smooth muscle mass (volume), calculated as medial cross-sectional area times V<sub>smc</sub> (normalized to vessel length) was increased by 56% (P < 0.05) in SHR.

Table 2

<table>
<thead>
<tr>
<th>Nuclear profiles</th>
<th>SMC volume density (V&lt;sub&gt;smc&lt;/sub&gt;)</th>
<th>Nuclear volume density (V&lt;sub&gt;nucl&lt;/sub&gt;)</th>
<th>Ratio, V&lt;sub&gt;smc&lt;/sub&gt;:V&lt;sub&gt;nucl&lt;/sub&gt;</th>
<th>Medial wall thickness (μm)</th>
<th>Medial x-section area (mm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Aortic length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHRs 13.6*</td>
<td>0.402 ± 0.010</td>
<td>0.024*</td>
<td>17.3*</td>
<td>117.9*</td>
<td>0.754*</td>
<td>0.304*</td>
</tr>
<tr>
<td>WKYs 19.0</td>
<td>0.426 ± 0.004</td>
<td>0.040</td>
<td>10.9</td>
<td>8.49</td>
<td>0.461*</td>
<td>0.195</td>
</tr>
</tbody>
</table>

Mean ± SEM.
* Significantly different from WKY (P < 0.05, t-test).
Analysis of Smooth Muscle Cell Ploidy (DNA Content) by Flow Microfluorimetry and Feulgen Microspectrophotometry

Flow cytometric results (Fig. 2) demonstrated that: (1) substantial numbers of tetraploid (4C) smooth muscle cells were present in both hypertensive and normotensive animals; (2) that the frequency of these cells increased as a function of age, although it appeared to plateau at five months of age in WKY rats; and (3) that the degree of polyploidism was from two to three times greater for SHR than WKY at 3 months of age and older. The incidence of cells in the G2 phase of the cell cycle, as determined by \(^{3}H\)-thymidine autoradiography, was 8.5 ± 3.5 (× 10\(^{-6}\)) and 4.5 ± 2.1 (× 10\(^{-6}\)) for SHR and WKY rats respectively (not significantly different), at 5 months of age, demonstrating that the high frequency of tetraploid cells did not represent the G2 component of a rapidly proliferating population of cells. Likewise, an extremely low S phase population was evident by flow microfluorimetry (Owens et al., 1981). It is important to note that flow cytometry uses curve-fitting procedures to estimate the number of cells with DNA contents between tetraploid and diploid. This value is used as an estimate of the number of cells in S, but is inaccurate for frequencies of replicating cells less than a few percent. Thus the autoradiographic data provide an important control and eliminate the possibility that any substantial portion of the population of cells in the G2 peak represents actively cycling cells.

A significant linear relationship existed between the frequency of tetraploid smooth muscle cells and animal age (Fig. 2). This was true for SHR at ages 1 month through 5 months (\(r^2 = 0.94, P < 0.001\)) as well as 1 month through 1 year (\(r^2 = 0.81, P < 0.001\)). For normotensive WKY, a significant linear relationship was present in animals up to 5 months of age (\(r^2 = 0.81, P < 0.001\). This correlation decreased substantially if 1-year-old animals were included (\(r^2 = 0.53, P < 0.01\)). In addition, a significant linear relationship was also found between the frequency of tetraploid smooth muscle cells and blood pressure (\(r^2 = 0.66, P < 0.001\)) in combined 3- and 5-month-old SHR and WKY rats (Fig. 3). This was also true when regression was calculated for SHR alone, (\(r^2 = 0.49, P < 0.02\), but not for WKY alone (\(r^2 = 0.130, not significant\). Inclusion of data from 1-year old animals into these regressions substantially reduced correlation coefficients [combined SHR and WKY (\(r^2 = 0.5, P < 0.001\)) SHR alone (\(r^2 = 0.05, not significant\); WKY alone (\(r^2 = 0.10, not significant\)]).

The more limited data in our initial report (Owens, 1981) was based only on isolated cells assayed by flow cytometry, microdensitometry, or biochemical determination of DNA content. This is subject to possible artifacts associated with isolation of cells. In the present study, measurements of the DNA content per smooth muscle cell nucleus were also made by Feulgen-DNA microdensitometry of tissue sections. This technique provides an important control to assess whether cell isolation techniques provide a representative cell sample, and gives information with regard to the location of polyploid cells in the vessel wall. Feulgen-DNA measurements in 5-month-old SHR and WKY rats showed distinct populations of diploid (2C) and tetraploid (4C) smooth muscle cells (Fig. 4). In addition, a small number (1.6%) of

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Frequency of tetraploid aortic smooth muscle cells in spontaneously hypertensive (SHR) and in normotensive Wistar-Kyoto (WKY) rats as determined by flow microfluorimetry. Nuclei were stained with diamidinophenylindole as described by Owens et al. (1981). SHR was significantly greater than WKY (\(P < 0.001\)) at 3 months of age and older. Means ± sem are shown.

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Linear regression of the frequency of tetraploid aortic smooth muscle cells to blood pressure for combined 3- and 5-month-old SHR and WKY rats (\(r^2 = 0.66, P < 0.001\)). The frequency of tetraploids was determined by flow microfluorimetry of DAPI-stained smooth muscle cell nuclei.
5 month SHR
72 64 56 48 40 32 24 16 8 0
Number of Smooth Muscle Cells/Nucleus

5 month WKY
72 64 56 48 40 32 24 16 8 0
Number of Smooth Muscle Cells/Nucleus

Feulgen-DNA (pg/nucleus)

FIGURE 4. Histograms of Feulgen-DNA measurements on aortic smooth muscle cells of 5-month SHR and WKY rats. Distinct populations of diploid (2C) and tetraploid (4C) smooth muscle cells were evident in both SHR and WKY. In addition, a small number of octaploid (8C) cells were present in SHR. We should point out that, since this method allows direct visual observation of each cell, the values represent the DNA content of individual nuclei.

octaploid (8C) cells were observed in SHR. The frequency of tetraploid cells, determined by microdensitometry on tissue sections, was 20.9 ± 2.2% and 8.7 ± 0.2% for 5-month SHR and WKY, respectively. The fact that these values were nearly identical to the values reported previously (Owens et al., 1981), based on Feulgen-DNA measurements on dispersed cells, and were very close to the results obtained by flow cytometric analysis of DNA on isolated cells (Fig. 2), provides good evidence that cell isolation methods did not preferentially select for or against polyploid cells. Although a detailed analysis of the distribution of polyploid cells in the vessel wall has been performed, they did not appear to be preferentially located in either the inner or outer portion of the wall.

To determine whether polyploidy was also present in venous smooth muscle, microdensitometric DNA determinations were also done on sections of the vena cava from 5-month animals. Tetraploid smooth muscle cells were present in vena cava of both SHR and WKY rats. The frequency of these cells (SHR = 5.7 ± 0.5%; WKY = 6.4 ± 2.9%), however, was not significantly different.

Simultaneous Evaluation of Smooth Muscle Cell Hypertrophy and Hyperplasty: Protein and DNA Determinations

To determine whether the hypertrophic smooth muscle cells were the polyploid cells, protein and DNA content was measured in individual cells by combined naphthol-yellow S and Feulgen-DNA microdensitometry. Results of protein-DNA measures (Fig. 5) showed that SHR diploid, tetraploid, and octaploid smooth muscle cells contain 36%, 136%, and 377% more protein per cell, respectively, than WKY diploids.

Utilizing the cell mass values in Figure 5 and the frequency of diploid, tetraploid and octaploid cells obtained by microdensitometry, the average smooth muscle cell mass was calculated for SHR and WKY cells.

Average cell mass WKY = 0.913(212.7) + 0.087(379.20)
= 227.2 pg protein/average cell

Average cell mass SHR = 0.775(290.3) + 0.209(502.7) + 0.016/1015.4 = 346.5 pg protein/average cell

This represents a 53% increase in average cell mass in SHR cells which is in relative agreement with the 61% increase in average cell mass based on measurements of actin content per cell that we previously reported (Owens et al., 1981). Importantly, this cellular hypertrophy can totally account for the 56% increase in aortic smooth muscle mass (0.304 vs. 0.195 mm$^3$/mm length, Table 2) which we measured by morphometry. Figure 6 illustrates the relative contribution of diploid, tetraploid, and octaploid cells to total smooth muscle...
cell mass in SHR and WKY thoracic aortas. From this it is apparent that the major portion of the increased smooth muscle mass in SHR was due to an increased frequency and size of polyploid cells.

Discussion

Studies by Folkow, Bohr, and others (Folkow et al., 1973; Lundgren et al., 1974; Berecek and Bohr, 1977) emphasize the importance of increased smooth muscle mass in elevation of vascular resistance in chronic hypertension. The data presented in this study and in a previous study (Owens et al., 1981) clearly establish that the difference in aortic smooth muscle mass between SHR and normotensive WKY can be accounted for by cellular hypertrophy with no detectable increase in smooth muscle cell number. The largest portion of this change in total smooth muscle mass is due to a 2- to 3-fold increase in the frequency of tetraploid and octaploid smooth muscle cells, which have 2.4 and 4.8 times, respectively, the mass of a WKY diploid cell. The remaining portion of the increase in smooth muscle mass is accounted for by a 1.4-fold increase in cell mass of SHR diploid cells.

Our results are in agreement with the morphometric data of Olivetti et al. (1980), who likewise found no evidence for an increase in cell number following subdiaphragmatic stenosis in rats and reported that the increase in aortic smooth muscle mass could be accounted for by a 55% increase in average smooth muscle cell volume. Importantly, they also reported an increase in average nuclear volume, suggesting a change in nuclear ploidy in this hypertensive model as well. Furthermore, we have observed increased smooth muscle cell ploidy in Goldblatt (two kidney, one clip) hypertensive rats (Schwartz et al., 1981). Thus, smooth muscle cell hypertrophy and hyperploidy are not unique to SHR, but appear to represent a common response in a variety of hypertensive models. While these results are in apparent contrast with previous studies (Crane and Dutta, 1963; Fernandez and Crane, 1970; Bevan, 1976; Bevan et al., 1976; Rorive et al., 1980), which have implicated increased smooth muscle cell number in thickened vessels, the present study demonstrates how an increase in DNA synthesis and content can occur without an increase in smooth muscle cell number.

The polyploid smooth muscle cells observed in the present study do not simply represent the G2 and mitotic compartments of a rapidly proliferating cell population. We know this because of the absence of a significant fraction of cells in the S phase of the cell cycle as seen by the very low frequency of labeled cells seen by [3H]-thymidine autoradiography (<10^-4). There are two other possibilities. First, these could be cells which, in the G0 or G1 phases of cell cycle, contain tetraploid (4C) DNA. Our observation of a small number of octaploid cells by cytospectrophotometry supports the possibility that at least some tetraploids are able to reenter S without undergoing mitosis. Alternatively, the cells with tetraploid DNA could be arrested in the G0 phase of the cell cycle and, upon an appropriate stimulus, would be able to enter mitosis without DNA synthesis. Arrest of cells in G0 has been reported in a number of tissues (Gelfant, 1962; Owen and MacPherson, 1963; Cameron and Cleffmann, 1964; Gordon and Lane, 1980).

The observation of a change in nuclear ploidy in association with cellular hypertrophy is not confined to hypertensive arterial smooth muscle. However, we should point out that, to our knowledge, the data presented here are the first definitive demonstration in vivo of the interrelationship between smooth muscle cell mass and ploidy, in which measurements were done on a cell-to-cell basis. Other studies are based on observations of changes in ploidy in association with organ or tissue hypertrophy. For example, hypertrophy of human uterine smooth muscle during pregnancy is associated with the appearance of polyploid smooth muscle cells (van der Heijden and James, 1975). Increases in nuclear volume, suggestive of a change in cell ploidy, have also been reported in hypertrophic smooth muscle in the ligated portal-anterior mesenteric vein (Berner et al., 1981) in urinary bladder (Brent and Stephens, 1975), colon (Brent, 1973), and in intestinal smooth muscle (Gabella, 1979a).

Changes in cellular DNA content and mass have also been reported for cells and tissues other than smooth muscle (Enesco and Puddy, 1964; Eisenstein and Wied, 1970; Carlson, 1973; Baserga, 1976; Rumyantsev, 1977; Sweeney et al., 1979). Observations in myocardium and skeletal muscle are particularly interesting. Physiological growth of both adult myocardium and skeletal muscle is thought to be limited to cellular hypertrophy (Carlson, 1973; Baserga, 1976; Rumyantsev, 1977). Loss of proliferative capacity in these cells has been equated with differentiation and...
attainment of contractile capability. In light of the similarity in the hypertrophic response of each of the muscle cells, it is interesting to speculate that the cellular hypertrophy and hyperploidy observed in this study may represent the normal growth response of a differentiated smooth muscle cell. This speculation may relate to observations of Chamley-Campbell et al. (1979), who found that differentiated contractile smooth muscle cells do not proliferate in primary culture until dedifferentiation occurs.

Our data do not provide any direct evidence relating to the stimulus producing smooth muscle cell hyperploidy, nor do they define whether changes in ploidy precede, parallel, or follow development of hypertension. Changes were not evident in prehypertensive 1-month-old SHR, but were present in 3-month-old animals which were already hypertensive. The demonstration of a significant relationship between ploidy and blood pressure suggests that increased pressure may be important. However, our data also suggest that hyperploidy and hypertrophy may represent a response to factors other than just increased pressure. For example, we noted tetraploid cells in veins subjected to very low pressure loads at a frequency approximately one-half that observed in normotensive arteries. Our data also show that cell ploidy and size increase in normotensive rats up to the age of five months. Studies by others have implicated local neural or humoral factors in control of vascular smooth muscle cell growth. Bevan and Tsuru (1981) have shown that sympathetic denervation of the ear artery in growing rabbits is associated with decreased smooth muscle cell DNA synthesis and vessel growth. Importantly, recent drug intervention studies (Rorive et al., 1980) suggest that changes in the mass of vascular wall cells in hypertension may be dissociable from pressure changes themselves. Rorive et al. (1980) examined the effects of captopril and reserpine treatment on DNA synthesis in uninephrectomized Goldblatt hypertensive rats. They found that both drugs were effective in preventing a rise in blood pressure, but only reserpine prevented increased smooth muscle cell DNA synthesis. In summary, the humoral, neural, mechanical, and developmental controls over cell ploidy and cell hypertrophy remain a largely unexplored area.

Our studies have focused on a large vessel because of the ease of analysis. There is, however, reason to suspect that similar changes occur in resistance vessels. Increases in wall thickness and smooth muscle mass have been reported in microvessels of hypertensive animal models (Ichijmak, 1969; Mulvany et al., 1978; Nordborg and Johansson, 1979; Warshaw et al., 1979) and man (Furuyama, 1962). Data of Mulvany, Halpern and co-workers (Mulvany et al., 1978; Warshaw et al., 1979) consistently show an increase in cross-sectional area of individual smooth muscle cells in mesenteric arteries of SHR, indicating that cellular hypertrophy is present. The possibility of DNA endoreplication at this site remains to be determined.

Whereas structural and functional changes in hypertension have usually been regarded as separate entities, there is reason to suspect that smooth muscle cell hyperploidy and hypertrophy may be associated with changes in functional capabilities. Hypertrophic intestinal smooth muscle cells have been shown to develop decreased contractile force per unit cross-sectional area (Gabella, 1979b). Whereas this change might represent some mechanical problem associated with cell size or shape (Gabella, 1979a), there is evidence for a decrease in the relative number of myosin filaments along with an increase in intermediate filaments (Berner et al., 1981), a noncontractile cytoskeletal protein. In light of the high frequency of polyploid cells in SHR, it is particularly interesting to speculate that changes in DNA content of hypertrophic cells might be related to some of the functional alterations reported for vascular smooth muscle in hypertensive animals (Holloway and Bohr, 1973; Jones, 1973; Hansen and Bohr, 1975; Lais and Brody, 1975). This could be important in the development of chronic hypertension, since changes in DNA, unlike changes in other macromolecules, are irreversible except by cell death. While we have not as yet determined whether smooth muscle cell hypertrophy and hyperploidy are reversible by conventional antihypertensive therapy, it has been shown that the polyploidy which develops in uterine smooth muscle during pregnancy does not reverse postpartum (van der Heijden and James, 1975). In addition, it has been demonstrated that during reversal of short-term myocardial hypertrophy, protein and RNA decrease, while DNA remains elevated (Beznak et al., 1969). The ploidy change observed in the present study may help to explain observations that antihypertensive treatment is not totally effective in reversing the structural component of increased peripheral resistance in animals with long-term hypertension (Lundgren and Weiss, 1979). The nuclear response to hypertension may thus represent a fixed change related to the establishment of a chronic hypertensive state.

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Address for reprints: Dr. Gary K. Owens, Department of Physiology, Box 449, University of Virginia, Charlottesville, Virginia 22908.

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G K Owens and S M Schwartz

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