Hyperplastic Growth Response of Vascular Smooth Muscle Cells Following Induction of Acute Hypertension in Rats by Aortic Coarctation

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SUMMARY. This study examines the growth response of vascular smooth muscle cells following induction of acute hypertension in rats by partial ligation of the abdominal aorta between the renal arteries. Systolic blood pressures proximal to the ligature increased dramatically within 3 days (from 135 ± 3 to 195 ± 7 torr) of surgery while pressures distal to the ligature were reduced from control values. The frequency of smooth muscle cells undergoing DNA replication was increased 25-fold in thoracic aortas of coarctation rats compared to sham-operated controls 9 days post-coarctation, while no differences were observed in cells in abdominal aortic segments 1 cm distal to the ligature. A small but significant increase in the frequency of tetraploid smooth muscle cells was observed in thoracic aortas of coarctation rats, but this increase accounted for less than 2% of the increase in medial DNA content. By far, the major growth response was hyperplasia, as evidenced by a 25% increase in thoracic aortic medial smooth muscle cell number without a change in mean cellular volume (μm³/cell) or mass (ng/cell). Whereas no evidence of endothelial denudation was observed in thoracic aortas of coarctation rats by scanning electron microscopy, endothelial cell turnover rates were increased 23-fold compared to controls, indicating that some form of endothelial "injury" or dysfunction was present. Consistent with this, morphological changes indicative of endothelial injury (e.g., subendothelial edema) were observed by light and transmission electron microscopy. The marked contrast between results of this study and our previous studies showing that aortic medial hypertrophy in spontaneously hypertensive and Goldblatt hypertensive rats was due to cellular hypertrophy and hyperploidy without hyperplasia, clearly demonstrates that the growth response of smooth muscle cells within a given blood vessel can be quite different, depending on the model of hypertension. It is suggested that a non-denuding form of endothelial "injury" may play an important role in the proliferative growth response of smooth muscle cells following induction of coarctation hypertension. (Circ Res 57: 695-705, 1985)

HYPERTENSION is well recognized as a major risk factor for development of atherosclerotic disease (Freis, 1969), but the mechanism whereby it exerts this effect is unknown. It has been suggested that the association between hypertension and atherosclerosis relates to the accelerated smooth muscle cell (SMC) growth which is a common feature in both diseases (Furuyama, 1962; Wolinsky, 1970; Bevan, 1976; Owens et al., 1981; Ross, 1981; Schwartz and Ross, 1984). This idea, however, is confounded: (1) by observations that in several slow-developing chronic models of hypertension (i.e., the spontaneously hypertensive rat and a two-kidney one-clip Goldblatt model) (Owens and Schwartz 1982, 1983; Olivetti et al., 1982), the major growth response in large arteries is SMC hypertrophy and hyperploidy (i.e., cell enlargement with DNA endoreplication), rather than hyperplasia which characterizes growth in intimal atherosclerotic lesions (Ross, 1981; Thomas and Kim, 1983; Barrett et al., 1983; Orekhov et al., 1983); and (2) by apparently conflicting reports regarding the nature of the SMC growth response in different experimental models of hypertension. For example, abdominal aortic stenosis-induced hypertension has been shown to elicit a rapid growth response of vascular SMC in thoracic aortas of a variety of animal species (Bevan et al., 1976; Bevan, 1976; Olivetti et al., 1980), but there is considerable controversy regarding the relative role of SMC hypertrophy vs. hyperplasia in this growth response. Bevan and co-workers (1976) studied SMC DNA replication in rabbits made hypertensive by partial constriction of the abdominal aorta just proximal to the superior mesenteric artery. They observed increased DNA synthesis and content in vessels proximal to the coarctation, which they interpreted as evidence for SMC hyperplasia. In contrast, Olivetti et al. (1980) found no increase in SMC number in thoracic aortas of rats following subdiaphragmatic constriction of the abdominal aorta, and concluded that medial thickening was due to SMC hypertrophy not hyperplasia. Whereas these conflicting results could be due to species differences, results in our laboratory (Owens...
et al., 1981; Owens and Schwartz, 1982, 1983) offered an alternative explanation, i.e., that the increase in DNA synthesis and content observed by Bevan et al. (1976) in an aortic coarctation model in rabbits in the absence of any increase in cell number in this same model in rats (Olivetti et al., 1980) could be accounted for by changes in SMC ploidy. An alternative possibility is that even subtle differences between hypertensive models may elicit quite different SMC growth responses due to differences in mechanisms of growth stimulation. This possibility is supported by recent studies from our laboratory (Owens, 1985) showing a differential effect of anti-hypertensive drug treatment on hypertrophic vs. hyperplastic SMC growth in the spontaneously hypertensive rat.

The observations cited above underscore the importance of distinguishing hypertrophic vs. hyperplastic SMC growth in defining the mechanisms for accelerated SMC growth in hypertension and for understanding the relationship between hypertension and atherogenesis. The objectives of the present investigation were: (1) to define the cellular nature of the SMC growth response following acute induction of hypertension in rats by aorta coarctation, and (2) to explore the possible role of endothelial alterations in the SMC growth response in this model.

Methods

Five-month-old male Sprague-Dawley rats (Hilltop) weighing 450–500 g were anesthetized with sodium pentobarbitol (Nembutal, Abbott Laboratories, 30 mg/kg, ip). The abdomen was opened with a midline ventral incision and a probe 0.40 mm in diameter held in contact with the wall of the abdominal aorta between the renal arteries. The aorta was ligated around the probe with surgical silk and the probe removed, leaving a constricted opening in the lumen equal to the diameter of the probe. Sham-operated animals (i.e., the ligature site on the vessel was manipulated in the same manner as in experimental animals) were used as controls. Blood pressure measurements were made under sodium pentobarbitol anesthesia via indwelling catheters in the left carotid and right femoral arteries at the time of sacrifice. In addition, blood pressure measurements in three coarctation rats were made 3 days after surgery to assess the rapidity of the blood pressure increase. Blood pressure measurements in conscious rats by conventional tail-cuff methods were not possible, since there was a large pressure drop across the ligature. However, we have observed (unpublished observations) that blood pressures of control Sprague-Dawley rats measured under sodium pentobarbitol anesthesia are not significantly different from those measured in conscious rats using a photoelectric tail-cuff pulse detector (ITTC).

Groups of rats were killed either 9 or 30 days after surgery so that we might study the acute vs. chronic effects of the coarctation procedure. Since our principal aim was to study coarctation as a model of hypertension-induced SMC growth, aortic segments analyzed in all studies were taken from sites at least 1 cm from the ligature to avoid possible effects of mechanical damage due to the surgery itself. Thoracic aortic segments included the region from the diaphragm to the descending arch of the aorta, whereas abdominal aortic segments were taken from the region between the inferior mesenteric artery (this was >1 cm distal to ligature) and the trifurcation.

Analysis of Smooth Muscle Cell Polyploidy and Cell Number

Rats were killed by CO₂ asphyxia, and a ventral incision was made to expose both the thoracic and abdominal aortas. The in situ length of the thoracic aorta was measured from the diaphragm to the beginning of the descending thoracic aorta. This same segment, as well as the abdominal aorta from the inferior mesenteric artery to the trifurcation, and the heart were excised, perfused with Hank’s buffer solution (GIBCO), and placed in fresh Hank’s buffer. Hearts were cleaned of loose connective tissue, dried for 48 hours, and weighed. After measuring the length of the thoracic aortic segment in vitro, an intimal-medial preparation was made by carefully dissecting away the adventitia and intercostal arteries as described by Wolinsky and Daly (1970). Wet weight determinations were then made on medial preparations. Similar dissections were done on abdominal aortic segments, and branches were removed. However, removal of adventitia from abdominal segments was much more difficult than from thoracic segments, and was not always complete, based on examination of light micrographs. The endothelium was scraped off with a piece of Teflon. Resultant medial preparations were used for subsequent isolation of vascular SMC for flow cytometric measurements of SMC DNA ploidy, and for evaluation of aortic medial DNA content and SMC number.

SMC nuclei were prepared for flow cytometric evaluation of DNA ploidy as previously described (Owens et al., 1981). In brief, SMC were enzymatically dissociated from medial preparations of thoracic or abdominal aortas, and nuclei were extracted and stained for DNA with diaminophenylindole. Measurement, acquisition, and analysis of DNA content was done on a FACS IV Fluorescent Activated Cell Sorter (Becton-Dickinson). Diploid and tetraploid compartments were estimated by an adaptation of the method of Dean and Jett (1974). Chicken red blood cells (2.5 pg DNA cell) were used as a standard.

For cell number determinations, thoracic aortic medial preparations were homogenized and DNA content was measured, using the fluorometric assay of Labarca and Peigan (1980). DNA content was expressed on the basis of vessel length (in vitro). SMC number was calculated by dividing aortic medial DNA content by the average DNA content per SMC determined by flow microfluorometric measurements. DNA and cell number determinations were not performed on abdominal aortic segments due to difficulties in removing the adventitia in these vessels.

Fixation

Rats were anesthetized with sodium pentobarbitol and perfusion fixed with 2% glutaraldehyde, 1% paraformaldehyde in Hank’s buffer, as previously described (Owens and Schwartz, 1981). Blood pressures were measured via a catheter in the left carotid artery, and fixation was carried out at the measured pressure. The fixed aorta then was excised and immersion fixed overnight. Adhering fat and connective tissue were dissected free, and segments were taken from multiple sites of the thoracic and abdominal aortic segments described above. Segments then were processed for: (1) autoradiographic determination of SMC and endothelial cell thymidine-labeling indices; (2) mor-
phometric analysis of thoracic aortic medial hypertrophy; and, (3) evaluation of endothelial integrity by scanning transmission electron microscopy.

[1H]Thymidine Autoradiography
Rats were given one dose of [1H]thymidine (New England Nuclear, 0.5 mCi/kg, 6.7 Ci/mg) intraperitoneally 1 hour before sacrifice. Fixed aortic segments were taken from three sites (i.e., the two ends and middle of thoracic and abdominal aortic segments, respectively) for determination of SMC-labeling indices. These were processed in JB-4 (Polysciences), and 1-μm cross-sections were cut and placed on glass slides. Slides were coated with NTB-2 emulsion (Kodak), and exposed for 2 weeks in the dark at 4°C. Slides were then developed in D-19, fixed with Rapid-Fix (Kodak), and stained in hematoxylin. The fraction of labeled SMC and intimal (endothelial) cells then was determined. A minimum of 10,000 SMC were counted from each animal.

Due to the low frequency of labeled endothelial cells and the limited number of endothelial cells present in cross-sections, endothelial labeling indices were also determined for thoracic aortas using modified Hautchen preparations as described by Schwartz and Benditt (1973). In brief, the luminal surface of fixed aortic segments was embedded in collodion. The remainder of the vessel then was stripped away, leaving a transparent sheet of endothelial cells. This preparation was dipped in Kodak NTB-2 emulsion and exposed for 2 weeks. The slides then were developed, stained with hematoxylin, and mounted under coverslips. The number of labeled endothelial cells and the endothelial cell density were measured with a light microscope equipped with a square reticle in the eye piece. At least 75,000 endothelial cells per animal were counted. The Hautchen procedure was not done on abdominal aortic segments because of technical difficulties associated with small vessel segments with many branches.

On some vessels, the labeled endothelial cells were identified by whole mount autoradiography using scanning electron microscopy (SEM) (Reidy and Schwartz, 1980). Pieces of the fixed vessel were opened longitudinally, pinned to Teflon, dried, and dipped in radiographic emulsion and processed, as described above. After development and fixation, the tissue was air dried and viewed by scanning electron microscopy.

Analysis of Aortic Smooth Muscle Mass and Content
Quantitative estimates of changes in aortic smooth muscle mass in coarctation and control rats were obtained either by: (1) determination of aortic medial wet weight for non-fixed specimens, or (2) morphometric analysis of medial cross-sectional area and smooth muscle volume density (i.e., VV/MC) on perfusion-fixed specimens (Owens and Schwartz, 1982). In brief, fixed aortic segments for morphometric evaluations were postfixed in osmium tetroxide for 2 hours, dehydrated, en bloc stained with 3% uranyl acetate, and embedded in Epon. Thick (1-μm) sections were cut and stained with toluidine blue for light microscopic evaluation of medial cross-sectional area. Thin sections for transmission electron microscopy were cut and post-stained with uranyl acetate and Reynold’s lead citrate. Medial cross-sectional area and vessel luminal diameter were determined by planimetry using a Zeiss Videoplan System. Multiple sections of the segments from each end of the thoracic aorta were evaluated. The average of these values was then taken as an estimate of the mean cross-sectional area of the thoracic aorta. SMC volume density (VV/MC) was determined on electron micrographs by standard point-counting techniques (Weibel and Bolender, 1973). A minimum of 15 electron micrographs (approximately 1325 μm²/micrograph) were analyzed from each animal. Using this sampling method, the variability between electron micrographs from individual animals (expressed as the standard error divided by the mean) was 3.4%. A quantitative estimate of thoracic aortic smooth muscle content was obtained by multiplying aortic medial cross-sectional area by VV/MC and by vessel length. Estimates of medial smooth muscle mass (i.e., wet weight per unit vessel length) were obtained by multiplying aortic medial wet weight (mg/cm length) by VV/MC.

Evaluation of Endothelial Integrity: Scanning Electron Microscopy
Aortic specimens were cleaned of periadventitial fat, opened longitudinally, and pinned onto pieces of Teflon. The tissue was dehydrated with increasing strengths of ethanol and critical point dried in liquid carbon dioxide. Pieces of the aorta were mounted on sticky copper tape and adhered to a SEM stub with conductive silver paint. Specimens were examined in a JEOL 35C microscope, at a voltage of 15 kV.

Statistical Analysis
Experimental results were evaluated by one-way analysis of variance. An alpha level of 0.05 was considered significant.

Results
Blood Pressure and Heart Weights
After partial constriction of the abdominal aorta between the renal arteries, blood pressure increased dramatically within 3 days in vessels proximal to the ligature, (i.e., carotid), but was reduced below normal levels in vessels distal (i.e., femoral artery) to the ligature (Table 1). Likewise, the mean systolic blood pressure was increased 49 torr compared to control at 9 days post-coarctation, but was reduced by 38 torr distal to the ligature. Increases in blood pressure with this model were transient, in that blood pressure proximal to the ligature was not different from controls in rats 30 days after surgery, although pressures distal to the ligature remained significantly lower than in normotensive controls.

Heart weights were not significantly different from controls in the 9-day post-coarctation group, but were increased significantly compared to controls in the 30-day post-coarctation group. Whereas heart weight:body weight ratios were elevated both 9 and 30 days after coarctation, the increase in the 9-day group was due in part to a 17% decrease in body weight in this group (presurgical weight = 487 ± 11; 9 days post-coarctation = 402 ± 14; 9-day control = 496 ± 10). This weight loss was temporary since body weights of the 30-day post-coarctation group were not significantly different from control.

Examination of the kidneys of rats at sacrifice showed that in both the 9-day and 30-day post-
Systolic Blood Pressure, Heart Weights, and Heart Weight:Body Weight Ratios of Aortic Coarctation-Induced Hypertensive and Normotensive Control Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Systolic blood pressure (torr)</th>
<th>Heart wt (g)</th>
<th>Body wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carotid artery</td>
<td>Femoral artery</td>
<td>(dry)</td>
</tr>
<tr>
<td>3-Day post-coarctation (n = 3)</td>
<td>195 ± 7*</td>
<td>90 ± 11†</td>
<td></td>
</tr>
<tr>
<td>9-Day post-coarctation (n = 16)</td>
<td>184 ± 8*</td>
<td>86 ± 9†</td>
<td>0.328 ± 0.007</td>
</tr>
<tr>
<td>9-Day controls (n = 10)</td>
<td>135 ± 3</td>
<td>124 ± 6</td>
<td>0.311 ± 0.013</td>
</tr>
<tr>
<td>30-Day post-coarctation (n = 11)</td>
<td>132 ± 10</td>
<td>82 ± 10†</td>
<td>0.374 ± 0.015‡</td>
</tr>
<tr>
<td>30-Day controls (n = 7)</td>
<td>143 ± 10</td>
<td>125 ± 9</td>
<td>0.340 ± 0.007</td>
</tr>
</tbody>
</table>

Results are expressed as means ± se. n = number of animals.
* Significantly greater than all other groups (P < 0.01, analysis of variance).
† Significantly less than all other groups (P < 0.01, analysis of variance).
‡ Significantly greater than controls (P < 0.01, analysis of variance).

coarctation groups, the right kidneys (proximal to the ligature) were markedly hypertrophied compared to the left kidneys. No renal necrosis was evident, based on gross visual examination.

Rates of Endothelial and Smooth Muscle Cell DNA Replication

Results of autoradiographic determinations of the frequency of SMC and endothelial cells undergoing DNA replication are presented in Figure 1. As expected, the frequency of [³H]thymidine-labeled cells were not different for the 9-day and 30-day control groups and were combined for statistical analyses. The frequency of SMC undergoing DNA replication was increased 25-fold (P < 0.001) in thoracic aortas of coarctation rats compared to controls 9-days after surgery. However, no increases were observed in SMC from abdominal aortic segment in these same animals (Fig. 1). SMC DNA replication rates were also increased 30 days post-coarctation, even though blood pressure had returned to normotensive levels at this time (Table 1).

Endothelial cell DNA replication rates were likewise increased dramatically (i.e., 23-fold, P < 0.001) compared to controls in thoracic aortas of rats 9 days following coarctation, whereas endothelial cell density was not significantly different (16.4 ± 1.0 vs. 16.2 ± 0.9 cells/100 μm² luminal surface area for 9-day post-coarctation and control rats, respectively). Endothelial cell replication rates appeared to be

![Figure 1: Frequency of smooth muscle cells (SMC) and endothelial cells undergoing DNA replication in aortic coarctation induced hypertensive (coarc.) and control rats as determined by [³H]thymidine autoradiography. Analyses were done on thoracic (open bars) and abdominal aortic segments (shaded bars) at least 1 cm from the ligature site (see Methods). Groups denoted with a "*" are significantly greater than control (P < 0.05, analysis of variance). In addition, in control animals SMC replication rates were significantly less in thoracic aortas than in abdominal aortas (marked with a "†") (P < 0.05, analysis of variance). Values are mean ± standard error of the mean. The number of animals evaluated (n) are indicated in parentheses.](http://circres.ahajournals.org/content/57/5/698/g3)
moderately increased from controls in thoracic aortas of rats 30 days post-coarctation, although differences were not statistically significant \((P = 0.12)\). Whereas large increases in endothelial replication rates occurred proximal to the ligature, no differences were apparent distal to the ligature. It should be noted that data for the abdominal aorta were obtained by analysis of aortic cross-sections, rather than endothelial Hauthens. Due to the small number of endothelial cells present in cross-sections (i.e., approximately 100/section), sample sizes were extremely small and valid statistical comparisons were not possible.

Determinations of internal aortic diameter on perfusion-fixed aortic samples showed no differences between controls and 9-day post-coarctation rats \((1.19 \pm 0.05\) vs. \(1.20 \pm 0.02\) mm). This finding, together with observations that thoracic aorta length was not different between 9-day post-coarctation (in vitro length = 3.01 \(\pm 0.05\) cm) and control (in vitro length = 2.98 \(\pm 0.06\) cm) groups, indicate that luminal surface area (i.e., luminal surface area = diameter \(\times\) length) was not altered in the 9-day post-coarctation group. Thus, the increased endothelial cell replication rate observed in 9-day post-coarctation rats was not associated with a change in endothelial cell number (i.e., since neither endothelial cell density nor luminal surface area was altered), but reflects increased endothelial cell turnover.

Although we did not quantify the frequency of adventitial cells undergoing DNA replication, it was apparent in autoradiographs that there was also a dramatic increase in the frequency of nonmuscle cells labeled with \([3H]\) thymidine in the adventitia of coarctation rats. This included fibroblasts as well as endothelial cells lining blood vessels located in the adventitia.

Evaluation of Smooth Muscle Cell Ploidy and Cell Number

To determine whether the increased SMC DNA replication was associated with hyperplasia or development of polyploidy, thoracic aortic medial SMC number and the fraction of polyploid SMC were evaluated. These analyses showed that SMC number (Table 2) was increased by 25% in thoracic aortas of 9-day post-coarctation rats compared to controls. Whereas there was also a significant increase in the fraction of tetraploid SMC, this could account for only a small fraction (1.6%) of the increase in aortic medial DNA content that occurred in 9-day post-coarctation rats. Neither SMC number, aortic DNA content, nor the fraction of tetraploid aortic SMC changed significantly between the 9th and 30th day after coarctation.

Consistent with the autoradiographic findings discussed above, no significant differences in the frequency of polyploid SMC were observed between abdominal aortas from coarctation and control groups, \((P > 0.1)\), demonstrating that changes in vessel DNA were limited to the proximal high-pressure region of the aorta. We did not analyze cell number or vessel DNA content in abdominal aortas, due to difficulties in separating the media from adventitia in these vessels and in obtaining reproducible abdominal aortic segments for analysis.

Assessment of Smooth Muscle Cell Hypertrophy vs. Hyperplasia

Whereas the preceding biochemical data clearly demonstrated that SMC number was increased in thoracic aortas of coarctation rats compared to controls, data did not distinguish whether cellular hypertrophy also may have contributed to aortic me-

### Table 2

<table>
<thead>
<tr>
<th>Percentage tetraploid SMC</th>
<th>DNA (pg)</th>
<th>DNA (pg)</th>
<th>DNA (pg)</th>
<th>SM C no. (\times 10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th o r a c i c aorta*</td>
<td>Abdom i nal aorta*</td>
<td>SMC</td>
<td>Aortic medial wet wt (mg)</td>
<td>Length (cm)</td>
</tr>
<tr>
<td>9-Day post-coarctation</td>
<td>7.1 (\pm 0.3)**</td>
<td>8.5 (\pm 0.6) *</td>
<td>7.10 (\pm 0.02)**</td>
<td>1.57 (\pm 0.07)</td>
</tr>
<tr>
<td>(n = 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-Day control</td>
<td>5.3 (\pm 0.3)**</td>
<td>7.0 (\pm 0.6) *</td>
<td>6.99 (\pm 0.02)**</td>
<td>1.57 (\pm 0.08)</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-Day post-coarctation</td>
<td>8.7 (\pm 1.3)**</td>
<td>8.2 (\pm 0.7) *</td>
<td>7.22 (\pm 0.08)**</td>
<td>1.57 (\pm 0.07)</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-Day control</td>
<td>5.5 (\pm 0.7)**</td>
<td>6.5 (\pm 0.9) *</td>
<td>7.00 (\pm 0.05)</td>
<td>1.43 (\pm 0.11)</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as means \(\pm\) se. \(n =\) number of animals.

* The thoracic aortic segment included the region from the diaphragm to the aortic arch. The abdominal aortic segment included the region from a point 1 cm distal to the ligature to the trifurcation.

† Significantly greater than controls \((P < 0.05,\) analysis of variance).
TABLE 3
Analysis of Thoracic Aortic Smooth Muscle (SM) Content, SMC Mass, and SMC Volume in Aortic Coarctation-Induced Hypertensive and Normotensive Control Rats

<table>
<thead>
<tr>
<th></th>
<th>SM mass (mg)</th>
<th>Medial cross-sectional area (mm²)</th>
<th>V&lt;sub&gt;SM&lt;/sub&gt; (Aorta)</th>
<th>SM content (mm&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>SMC mass (ng/cell)</th>
<th>SMC volume (μm&lt;sup&gt;3&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-Day post-coarctation</td>
<td>4.84 ± 0.18*</td>
<td>0.55 ± 0.02*</td>
<td>0.353 ± 0.008</td>
<td>5.93 ± 0.32*</td>
<td>1.72 ± 0.08</td>
<td>636 ± 40</td>
</tr>
<tr>
<td>n = 13</td>
<td>n = 9</td>
<td>n = 9</td>
<td></td>
<td>n = 6</td>
<td>n = 6</td>
<td></td>
</tr>
<tr>
<td>9-Day control</td>
<td>3.98 ± 0.11</td>
<td>0.48 ± 0.02</td>
<td>0.356 ± 0.010</td>
<td>4.97 ± 0.24</td>
<td>1.87 ± 0.05</td>
<td>699 ± 34</td>
</tr>
<tr>
<td>n = 7</td>
<td>n = 7</td>
<td>n = 7</td>
<td></td>
<td>n = 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE. n = number of animals.
* Significantly greater than 9-day control (P < 0.025, analysis of variance).

dial hypertrophy in this model. This question was addressed by: (1) determining what fraction of the total increase in aortic medial smooth muscle content or mass in 9-day post-coarctation rats could be accounted for by increased cell number without a change in individual cell mass, and (2) calculating mass/SMC (i.e., smooth muscle mass/cm + SMC number/cm = mass/SMC) and volume/SMC (i.e., smooth muscle content/thoracic aorta + SMC number/thoracic aorta = volume/SMC).

Results demonstrated (Table 3) a 21.6% increase in aortic medial smooth muscle mass (mg/cm) and a 19.3% increase in smooth muscle content (mm<sup>3</sup>/thoracic aorta) in 9-day post-coarctation rats compared to normotensive controls. Thus, the increase in aortic medial SMC number (i.e., 25%, Table 2) could account for the increase in smooth muscle content or mass without a change in individual cell size. This conclusion was directly confirmed by observations that mean SMC volume (μm<sup>3</sup>/cell) and mass (ng/cell) were not significantly different between coarctation and control rats (Table 3). In fact, mean values were slightly lower in coarctation rats than in controls. Note that SMC volumes reflect relative values based on analysis of fixed dehydrated specimens which have undergone shrinkage, and thus they cannot be compared directly to SMC mass values obtained from hydrated specimens.

Evaluation of Endothelial Integrity

The dramatic increase in endothelial turnover rates in 9-day post-coarctation rats (Fig. 1), clearly implied that some form of endothelial injury or dysfunction may be present. To explore further the nature of this "injury," endothelial morphology was examined in light micrographs used for autoradiography, and by scanning and transmission electron microscopy (Figs. 2 and 3).

No evidence of frank endothelial denudation was seen by scanning electron microscopy (Fig. 2) in either the 9- or 30-day post-coarctation groups. In addition, no platelets or leukocytes were evident on the endothelial surface. Whereas gross denudation was not evident by scanning electron microscopy, alterations suggestive of endothelial dysfunction or injury were observed by transmission electron microscopy (Fig. 3) in thoracic aortic segments from hypertensive rats. The most consistent findings were a thickened and edematous intima characterized by a widened subendothelial space that contained...
FIGURE 3. Transmission electron micrographs (panels a and b) of the thoracic aorta of rats 9 days after aortic coarctation. The endothelium (E) is continuous, with no breaks, but the subendothelial space (SES) appears thickened and contains large amounts of amorphous material. In addition, an intimal cell (presumably a modified smooth muscle cell) is evident in the subendothelial space in panel b. IEL—internal elastic lamina. Panel c shows a micrograph from the thoracic aorta of a control rat for comparative purposes. Vessels from nine 9-day post-coarctation rats, five 30-day post-coarctation rats, and seven control rats were examined. Approximately 25 electron micrographs were examined from each vessel. Magnification bar = 1.0 μm.
much granular material, and occasional mononuclear cells (Fig. 3). Interestingly, these changes were observed in some samples but not others within the same vessel, suggesting that changes were focal in nature. The precise anatomical location of such changes was not evaluated. Intimal SMC were relatively rare in the 9-day post-coarctation group (Fig. 3b), but were observed frequently in the 30-day post-coarctation group. This observation was, for the most part, restricted to small numbers of cells at focal sites. Taken together, these morphological data and endothelial cell turnover data (Fig. 1) suggest that some form of non-denuding endothelial "injury" (or dysfunction) was present in aortic regions proximal to the coarctation site.

Discussion

The studies presented here have focused on exploring the growth response of smooth muscle cells in a large vessel to an acute rise in blood pressure in an attempt to understand better the role of hypertension as a risk factor for atherogenesis. The results of the present study demonstrate that the principal SMC growth response following induction of coarctation hypertension in the rat is SMC proliferation. Although we saw a very modest increase in the fraction of polyplloid SMC, this increase accounted for less than 2% of the increase in thoracic aortic medial DNA content, and no changes in SMC volume or mass were observed. Consistent with these findings, Bevan and co-workers (Bevan, 1976; Bevan et al., 1976) observed an increased frequency of mitotic SMC following aortic coarctation-induced hypertension in rabbits, indicating that SMC proliferation occurs in this model, although their data did not define the relative contribution of cellular hypertrophy vs. hyperplasia. The proliferative response of SMC following induction of coarctation hypertension is in marked contrast to our previous observations showing that aortic medial hypertrophy in spontaneously hypertensive and Goldblatt hypertensive rats was due principally to cellular hypertrophy and hyperplody, not hyperplasia (Owens et al., 1981; Owens and Schwartz 1982, 1983; Owens, 1985). Taken together, the studies cited above demonstrate that the growth response of vascular SMC within a given blood vessel can be quite different in different models of experimental hypertension. Furthermore, the fact that results of our studies and those of Bevan (1976) appear to be in direct contrast to those of Olivetti et al. (1980), which showed a strictly hypertrophic SMC growth response following aortic coarctation in rats, indicate that differences within a given hypertensive model may also modify the SMC growth response. Note that: (1) the rats utilized by Olivetti et al. (1980) were much younger and a different strain than ours (4-week Wistar rats vs. 5-month Sprague-Dawley rats); (2) the degree of aortic constriction was much greater in our studies than theirs (i.e., we used a 0.400 mm ligature in a 5-month-old rat, whereas they used a 0.450 mm ligature in a 4-week-old rat); and (3) coarctation was done between the renal arteries in our studies, but above both renals in their studies. Direct comparisons of blood pressure changes are not possible, since these were not measured by Olivetti et al. (1980).

Observations that aortic SMC undergo quite different growth responses in different hypertensive models imply that the signals for growth in cell size as opposed to growth in cell number are different. This idea is also supported by recent studies in our laboratory (Owens, 1985) showing a differential effect of antihypertensive drug treatment on SMC hypertrophy vs. hyperplasia in the SHR (i.e., lowering of blood pressure by drug treatment prevented smooth muscle cell hypertrophy and hyperplody but not hyperplasia between 5 and 7 months of age). Whereas the studies presented here do not define the specific mechanisms responsible for hypertrophic vs. hyperplastic SMC growth in different hypertensive models, we believe they do provide important insight in this area. A major difference between the coarctation model examined here, compared to hypertensive models characterized by SMC hypertrophy and hyperplody (Owens and Schwartz, 1982, 1983), is that accelerated SMC growth was accompanied by a dramatic increase in endothelial cell turnover. (Note that our discussion here must a priori be limited to those models where the role of SMC hypertrophy vs. hyperplasia has been clearly defined and where endothelial data are available in the same vessel in the same model.) In adult animals, aortic endothelial cells turn over at an extremely low rate, and there is considerable evidence that any increase in turnover is indicative of cell injury (Schwartz and Benditt, 1977; Haudenschild and Schwartz, 1979; Reidy and Schwartz, 1981, 1984; DeChastonay et al., 1983). In fact, since endothelial cells are so adept at repairing small injuries, and can do so without any obvious breaks in the endothelial monolayer, a sudden increase in endothelial cell turnover is perhaps one of the few techniques capable of detecting vascular injuries which may not be observed by standard morphological techniques (Reidy and Schwartz, 1983, 1984). Consistent with turnover data, observations of subendothelial edema in regions proximal to the coarctation site in the present studies are also indicative of endothelial "injury" or dysfunction. In addition, more extensive morphological evidence for endothelial "injury" in this model has been reported previously by Huttner et al. (1970), who studied intimal changes and uptake of macromolecular tracers in rats made hypertensive, by a coarctation procedure very similar to ours. In contrast to these observations in coarctation hypertension, endothelial cell replication rates are not increased in SHR, as opposed to WKY (Schwartz and Lombardi, 1982), and aortic intimal changes consistent with endothelial injury are virtually absent except in older animals (Limas
et al., 1980; Haudenschild et al., 1981; Owens and Schwartz, unpublished observations). It is of interest, that the time of appearance of intimal changes in SHR reported by Limas et al. (1980) (i.e., 20 weeks) coincides with the time period during which some aortic SMC hyperplasia is detectable (Owens, 1985). Similar to findings in SHR, no endothelial alterations were apparent by transmission electron microscopy or \(^{3}H\)thymidine autoradiography in our studies of two-kidney one-clip Goldblatt hypertensive rats (unpublished observations). Schwartz and Benditt (1977) did observe a small transient increase in endothelial replication in Goldblatt hypertensive rats, however their model involved immediate occlusion of the renal artery, and onset of hypertension was more acute and severe than in our studies (Owens and Schwartz, 1983).

The observations cited above provide evidence that some form of non-denuding endothelial "injury" or dysfunction occurs in coarctation hypertension that is not generally present in hypertensive models characterized by aortic SMC hypertrophy and hyperplasia. There is compelling evidence suggesting that this endothelial injury could contribute to the SMC proliferative response observed in the present studies by a variety of mechanisms. (1) Cultured endothelial cells produce several growth factors for SMC, including endothelial-derived growth factor (Gajdusek et al., 1980) and a platelet-derived growth factor (PDRF)-like protein (DiCorleto and Bowen-Pope, 1983). Furthermore, recent studies by Barrett et al. (1984) using sis oncogene probes provide strong evidence that the PDGF-like growth factor is produced by endothelial cells in vivo. Of particular interest to the present studies are recent observations that production of the PDGF-like factor by cultured endothelial cells is increased in response to injury (Fox and DiCorleto, 1984). (2) It has been well-documented in studies of experimental atherogenesis that deliberate endothelial denudation can elicit a SMC proliferative response (Spaet et al., 1975; Ross, 1981), perhaps through growth factors derived from platelets or monocytes adherent to the injured wall (Schwartz and Ross, 1984). Although neither platelets nor monocytes were observed in the present studies, we cannot exclude a role for these cells, since our morphological data do not eliminate the possibility of a transient interaction of platelets or monocytes with the vessel wall with release of growth factors. Indeed, Reidy and Schwartz (1983) have presented evidence of platelet interaction with the vessel wall, despite the presence of an intact endothelium, following endotoxin-induced endothelial injury in rats. (3) Increased influx of blood-borne mitogenic factors may occur as a result of injury-induced increases in endothelial cell permeability (Ross, 1981; Schwartz and Ross, 1984). It is worth noting that the increased proliferation of adventitial fibroblasts following coarctation in this and previous studies (Fernandez and Crane, 1969; Bevan, 1976) is consistent with a role for endothelial injury in the growth response, since each of the growth factors cited above also stimulates fibroblast growth. Significantly increased growth of adventitial cells does not occur in aortas of SHR (unpublished observations). Whereas the preceding considerations support a possible role for endothelial "injury" in the SMC proliferative response observed in the present studies, evidence is largely circumstantial, and more direct evidence is needed. In addition, we can only speculate on why this model of hypertension induces endothelial "injury" whereas several others do not. One possibility is that the sudden and dramatic increase in blood pressure following coarctation causes hemodynamic injury. In contrast, such injury may not occur in models characterized by SMC hypertrophy (i.e., SHR (Owens and Schwartz, 1982) and two-kidney one-clip Goldblatt (Owens and Schwartz, 1983) where hypertension develops over a period of weeks or months. Observations suggest that the rate of pressure increase may be an important determinant of the nature of the SMC growth response.

An alternative mechanism which must be considered to explain the coordinate increases in proliferation of medial SMC, intimal endothelial cells, and adventitial fibroblasts, is that some form of generalized vessel "injury" occurs in this model as a result of an acute increase in wall stress, or that the acute increase in wall stress itself is the stimulus for proliferation. However, the possible cellular mechanisms which could mediate such a response are unknown. In addition, no evidence of generalized vessel injury was observed by transmission electron microscopy, although it is unclear whether such changes could be detected morphologically. Several observations appear to be inconsistent with a direct role of increased wall stress in the proliferative response: (1) SMC replication remained elevated even after blood pressure and wall stress had returned to normal (i.e., 30 days post-coarctation); and (2) Fernandez and Crane's (1969) observations of increased replication in non-smooth muscle cells, including fibroblasts and renal tubular epithelial cells adjacent to affected arteries, are more consistent with a role for locally generated humoral factors in the growth response rather than wall stress per se. However, it is possible that an acute increase in wall stress is responsible for activation or release of growth factors. Note that an abrupt increase in wall stress probably does not occur in the SHR because changes in vessel mass and wall stress occur in parallel over a relatively long time period (Owens, 1985).

Finally, it is important that we comment on the possible significance of our findings to studies of atherosclerosis. Previous studies implicating endothelial injury or dysfunction in initiation of SMC proliferation in atherogenesis are based principally on studies of deliberate injury such as balloon angioplasty (Spaet et al., 1975; Schwartz et al., 1975) or air-drying (Fishman et al., 1975), which result in total endothelial denudation (for a further discussion
of what constitutes endothelial "injury," see reviews by Schwartz and Ross (1984) and Reidy and Schwartz (1984)]. These injury models have several major disadvantages with regard to defining the role of endothelial injury in atherogenesis. (1) The degree of endothelial injury is not physiological, and is not likely to occur under any circumstances in vivo. (2) The method of injury is likely to damage wall components other than endothelium, raising the possibility that SMC proliferation represents a response to something other than endothelial damage. This possibility is supported by recent observations by several groups (Hirsch and Robertson, 1978; Reidy and Schwartz, 1981) showing that when a thin nylon string is used to reduce trauma to the vessel, removal of small areas of endothelium does not induce SMC proliferation despite endothelial loss and platelet adherence. (3) The role of endothelial cells themselves, which are a very potent source of SMC mitogens (Gajdusek et al., 1980; Di Corleto and Bowen-Pope, 1983), cannot be studied using these denudation models. In contrast, the data presented here have demonstrated that some form of non-denuding injury or dysfunction occurs after induction of acute hypertension by aortic coarctation, and that this is accompanied by a SMC proliferative response. Unlike other "injury" models of atherogenesis, it is not likely that the SMC proliferation observed in these studies results from direct mechanical injury to the vessel during surgery. Furthermore, the degree of endothelial injury or dysfunction associated with coarctation hypertension is probably more relevant to what might occur in human hypertension, since available evidence suggests that detectable endothelial denudation does not occur (Schwartz and Ross, 1984). The coarctation model described here should thus be a very useful model for exploring mechanisms of SMC growth initiation. Finally, results of this study support the hypothesis that the increased prevalence of atherosclerotic disease in hypertensives (Freis, 1969) may be the result of accelerated SMC proliferation secondary to some form of non-denuding endothelial injury. Significantly, our results demonstrate that even relatively short-term increases in blood pressure, if severe in nature, can result in a dramatic increase in SMC number. This observation is significant, since it suggests that even transient hypertensive episodes may contribute to accelerated atherogenesis.

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