Changes in Size and Shape of Smooth Muscle Cells from the Portal Vein of Spontaneously Hypertensive Rats: An Ultrastructural Analysis*

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Summary. The portal vein was investigated in male spontaneously hypertensive rats (SHR), from Wistar-Kyoto (WKY) and Wistar strains, in animal 16-20 weeks old. In SHR, the inner circular smooth muscle was unchanged, but the outer longitudinal layer showed marked alterations in shape and size, readily observed in three-dimensional micrographs using scanning electron microscopy. The cells in both Wistar and WKY were elongate and tubular with little variation along their lengths and with a relatively smooth sarcolemma. This applied to both the inner and outer layers of smooth muscle. In contrast, the smooth muscle cells from SHR in the outer layer varied considerably in thickness along their lengths, and had very irregular outlines with numerous pits or depressions of varying sizes. In addition, the cells frequently had major forks or branches. The vasa vasorum running through the muscle layer, fibroblasts and nerve bundles were also indentified. Sectioned material (transmission electron microscopy) showed a change in shape and hypertrophy of the smooth muscle cells from the portal vein of SHR, and also demonstrated a significant increase in paracellular connective tissue in the outer layer of smooth muscle. Such major morphological alterations in the outer layer of smooth muscle in the portal vein from SHR could have profound effects on functional studies.

The portal vein represents a portion of the vascular network that is not subjected to the same elevations in blood pressure developing on the arterial side in spontaneously hypertensive rats (SHR), compared with their Wistar-Kyoto (WKY) controls (ARNER and HELLSTRAND, 1981; SHIMAMURA et al., 1989). It is a vessel that has been used extensively in physiological and pharmacological studies on vascular smooth muscle, and all aspects of its characteristics have been recently reviewed (Sutter, 1990). The rat portal vein has two layers of smooth muscle: an inner circular and an outer, much thicker, longitudinal layer (JOHANSSON et al., 1970; TSAO et al., 1970; LJUNG et al., 1979). A number of reports have demonstrated that the longitudinal and circular layers vary considerably in their responses (SUTTER, 1990). Few reports, however, have described the morphology of the vessel from hypertensive animals. If there are significant changes in the size or shape of the smooth muscle, this could affect such data as the responsiveness of the tissue to pharmacological agents. Available information is contradictory: changes in the muscle mass of SHR have been described (SUTTER and LJUNG, 1977; GREENBERG et al., 1978, 1981), but no morphological changes were found in the portal vein from SHR when examined by MULVANY and coworkers (1980).

This report describes the ultrastructural details of the wall of the portal vein from SHR, WKY and Wistar animals, using both scanning and transmission electron microscopy (SEM, TEM).

MATERIALS AND METHODS

Portal veins were obtained from male inbred SHR, WKY and Wistar rats maintained in the Department of Anatomy. The animals were housed (two per cage in a controlled environment with water and Purina Laboratory Chow ad libitum. The tissue described in this investigation came from animals 16 to 20 weeks old. Six to 10 animals from each of the three strains were assessed for each measurement or morphological criterion, with a minimum of three to five

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samples averaged from each animal.

**Blood pressure measurements**

The arterial and venous blood pressures were recorded in ether anaesthetized animals simultaneously using two Statham pressure transducers connected to a precalibrated Grass Polygraph or directly to a water manometer, respectively. Arterial (systolic, diastolic and mean) pressures were measured via a cannula in the left common carotid artery, and venous pressures via a cannula in the portal vein.

**Fixation and processing**

SEM: After blood pressures were recorded, a short length of polyethylene tubing (PE50) was inserted into the portal vein and ligatures were placed at its entrance to the hilum of the liver and approximately 7 mm distally. This ensured the orientation of the vein, preventing it from curling up or twisting, and allowed easy manipulation by holding the tubing during processing. The veins were placed in lactated Ringer's solution at 37°C. Enzymatic and chemical methods to remove the extraneous tissue to expose the smooth muscle cells were similar to those used previously for smooth muscle (BALUK and GABELLA, 1987; KRIZMANICH and LEE, 1987). The Ringer's solution had 2.5 mg/ml of collagenase Type II (385 Units/mg) and 2.5 mg/ml trypsin (8460 BAEE Units/mg), and the veins were left for 1 h with gentle agitation. Following rinsing with Ringer's, the veins were fixed at 20°C for 2 h in 3% glutaraldehyde in Ringer's solution. Samples were again rinsed, and then placed in 8N hydrochloric acid at 68°C for 25 min. After a final rinse, the samples were dehydrated in a graded series of alcohol, substituted with amyl acetate, and subjected to critical point drying using CO2. They were mounted on aluminum stubs with silver print and sputter coated with a thin layer of gold. Assessment and photography were carried out using a Cambridge Stereoscan S-4 SEM. Measurements of the maximum width of cells were taken directly from micrographs.

TEM: All veins were obtained from animals that were perfusion fixed at each animal's mean blood pressure via the cannula in the common carotid artery. The methods have been described in detail previously (TODD et al., 1983; TODD, 1990; TODD and GOWEN, 1991). Following routine processing and embedding, semithin (0.5μm) sections were cut for light microscopy, and ultrathin (80 nm) sections for TEM. Measurements of the wall and luminal areas were obtained from averages of complete cross sections,

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**Table 1. Blood pressures (both arterial and venous) in 16 to 20-week-old SHR, WKY and Wistar Rats.**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Arterial (mm Hg)</th>
<th>Venous (cm H2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td>WKY</td>
<td>131.2±3.0</td>
<td>97.3±2.0</td>
</tr>
<tr>
<td>Wistar</td>
<td>137.8±4.1</td>
<td>103.0±2.8</td>
</tr>
<tr>
<td>SHR</td>
<td>184.4±7.6**</td>
<td>113.3±5.8*</td>
</tr>
</tbody>
</table>

**p≤0.01, *p≤0.05. Significant differences between SHR and WKY or Wistar.**

**Table 2. Results from analyses of micrographs of the portal vein for vessel size, the amount of cellular (smooth muscle, S. M.) to noncellular (connective tissue, C. T.) components in the outer longitudinal layer of the tunica media, and smooth muscle dimensions.**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Areas of vessel in mm×10⁻³</th>
<th>Cell to noncell%</th>
<th>Maximum S. M. width in μm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wall</td>
<td>Lumen</td>
<td>S. M.</td>
</tr>
<tr>
<td>WKY</td>
<td>228±16</td>
<td>4316±624</td>
<td>84.6±3.4</td>
</tr>
<tr>
<td>Wistar</td>
<td>212±15</td>
<td>4713±580</td>
<td>79.3±2.1</td>
</tr>
<tr>
<td>SHR</td>
<td>221±10</td>
<td>5102±687</td>
<td>56.7±8.3**</td>
</tr>
</tbody>
</table>

**p≤0.01, *p≤0.05. Significant differences between SHR and WKY or Wistar.**
photographed using a Leitz Orthoplan microscope, and measured using a digitizing tablet and a software program run on the University Mainframe (AMDAHL 471/V8) Computer. All details of the composition of the wall from sections were obtained using either a Philips 300 or 301 TEM. Measurements of the width of the cells were made using the above software, and evaluation of the amount of paracellular connective tissue was made using point counting to give the proportion of cellular to noncellular components in the outer layer of muscle (TodD et al., 1983, 1989; TodD and Gowo, 1991).

Statistics
Values are given as mean ± standard error of the mean (M±SE). Differences were considered statistically significant when assessed using Analysis of Variance (ANOVA) at the p≤0.01 or p≤0.05 level.

RESULTS
The simultaneous measurements of arterial and venous blood pressures showed significant elevation on the arterial side only in SHR, but no differences in pressure were present within the portal vein (Table 1). There also were no differences in the overall size of the portal vein either in wall or luminal areas (Table 2). However, there were marked differences in the smooth muscle cells of the outer longitudinal layer in veins from SHR, but not in the inner circular layer. This response in the outer layer can only be readily observed in samples where both layers are visible in three dimension in the same micrograph, when each layer has been individually photographed, at the same magnification, from the same sample (Fig. 1) In all SEM photographs, the long axis of the vein runs horizontally across the page. In portal veins from WKY rats, the outer longitudinal smooth muscle cells have a fairly regular or even surface and resemble the cells of the inner layer, being elongate or tubular (Fig. 2). Wistar animals also showed similar characteristics of the smooth muscle to that present in WKY, with regular outlines and an overall orderly arrangement of cells in parallel array (Fig. 3).

TEM observation of sectioned tissue confirmed the SEM findings. The smooth muscle cell profiles of inner and outer layers were similar in WKY, whereas the hypertrophied profiles of smooth muscle cells with very irregular outlines were present in the outer
layer only, in portal veins from SHR (Fig. 4). The differences in cell width were confirmed from measurements of the cells (Table 2). Processing for SEM resulted in more shrinkage of the tissue; the smaller widths of the smooth muscle cells reflect this, while still showing the differences between the normotensive and hypertensive strains.

The outer smooth muscle cells from the portal vein of SHR were greatly altered (Table 2). Their appearance showed changes in shape and size. The cells were no longer tubular but had a complete change in shape, hypertrophying and becoming much more irregular, and tending to have a thicker portion rather than being relatively uniform along their length (Figs. 1, 2). The sarcolemma, rather than being relatively smooth, had folds, pits, or depressions throughout the whole surface of the cells. They also had branches or forks (Fig. 5).

Other components of the wall could also be identified in the micrographs. Blood vessels (vasa vasorum) were seen coursing through the muscle layer (Figs. 3, 4, 6). In addition, smooth surfaced stellate fibroblasts were observed (Fig. 7), and components of the autonomic plexus were present (Fig. 8). Tributaries of the portal vein also had outer longitudinally arrayed smooth muscle (Fig. 9).

Since connective tissue was removed in the processing for SEM, it was not possible to assess the

Fig. 2. Details of the longitudinal smooth muscle cells (L) in the portal vein from SHR (a) and from WKY (b). The cells from SHR have a very irregular sarcolemma with numerous pits or depressions. In WKY the cells are much more uniform along their length and the sarcolemma is smoother. Bar: 5 μm.
differences, although the samples from WKY and Wistar rats had much more uniformity than those from SHR. In sectioned vessels, there were minimal amounts of connective tissue between the cells, with the cells evenly distributed. The proportion of para-cellular connective tissue in the outer muscle layer was much more heterogeneous in veins from SHR (Fig. 4). Point counting revealed a significant elevation of the connective tissue in the outer layer in veins from SHR with the accompanying drop in proportion of the smooth muscle (Table 2).

**DISCUSSION**

Previous reports on changes in the rat portal vein from hypertensive animals have been controversial. The increased overall mass of the longitudinal muscle with increased stress in response to agonists has been documented by Sutter and Ljung (1977) when SHR were compared with Wistar animals. In stroke prone SHR, outer longitudinal smooth muscle areas were increased as were the amplitude and duration of spontaneous contraction of the vein, over that found with WKY (Shimamura et al., 1989). Numerous investigators have shown increased physiological and pharmacological responses in SHR over WKY (Arner and Hellstrand, 1981; Pegram and Ljung, 1981; Pinelis et al., 1987; Sutter, 1990). Other reports in which individual cells in the outer layer of the portal vein were evaluated have confirmed hypertrophy of smooth muscle cells in 6-month-old animals.

![Fig. 3. Longitudinal smooth muscle cells (L) from the portal vein of a Wistar animal. The cells resemble those found in WKY rats. A blood vessel (BV), probably a venule, is coursing through the bundles of smooth muscle cells. Bar: 5 μm.](image-url)
(GREENBERG et al., 1978, 1981). In contrast, MULVANY et al. (1980) found no differences of any sort in morphological or contractile properties of portal veins from SHR or WKY, but their animals at 3-4 months were younger than in the other reports. It is possible that major changes begin to occur only in animals 4 months (16 weeks) or older.

The functional differences shown between the inner circular and outer longitudinal smooth muscle (SUTTER, 1990) have their counterpart here, in that morphological changes occurred in the outer layer only in SHR (Figs. 1, 4). It is not clear why the cells show such dramatic changes since there is no elevation of the blood pressure to cause a direct effect. The changes that occur, however, are similar to those which occur on the arterial side with hypertension, with hypertrophy of the smooth muscle and an increase in the connective tissue (TODD, 1992). It has been suggested that a circulating humoral factor in SHR causes the alterations in the smooth muscle of

**Fig. 4.** Cross section through the wall of the portal vein from WKY (a) and from SHR (b and c). The circularly arranged smooth muscle (C) next to the endothelium is similar in the three sections. The lumen is in the upper right hand corner of each micrograph. The two sections from SHR (b and c) show the variation in amount of connective tissue as well as the hypertrophy and very irregular profiles typical of the longitudinal layer of smooth muscle. This layer (L) from WKY (a) has cells similar in structure to the circular smooth muscle (C). Bar: 2 μm.
the portal vein since veins from WKY parabiosed to SHR show similar hypertrophy and contractile responses to those of SHR (Greenberg et al., 1981).

The results in this report show that, although there were no differences in overall wall areas in portal veins from SHR, the longitudinal smooth muscle cells are markedly changed in size and shape when examined in three dimensions using SEM. These smooth muscle cells have previously been shown by us to have significantly increased surface area to volume ratios in portal veins from SHR (Todd et al., 1989), which is confirmed by the three dimensional appearance reported here. Changes in shape are very difficult to quantify in irregularly shaped cells, and we have explored the possibility of using fractal dimensions. The degree of surface irregularities can be determined using fractals (Mandelbrot, 1983), and thus to evaluate changes in smooth muscle shape as is demonstrated here. This type of analysis when applied to the sectioned smooth muscle cells of the portal vein showed that the cells from the control animals had a significantly more uniform profile than those from SHR (Todd, 1990).

The increase in size and the much more irregular shape we have demonstrated for smooth muscle cells from the portal vein of SHR as well as the increase

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**Fig. 5. a and b.** Examples of longitudinal smooth muscle cells (L) from the portal vein of SHR. As well as showing the irregularities of these cells, the major forks or branches that occur are indicated (B). Bar: 2 µm.
Fig. 6. A blood vessel coursing through the longitudinal smooth muscle (L) in a portal vein from SHR. The endothelial cells (E) are partially separated due to the processing of the tissue. The basal lamina (B) remains in some areas and red blood cells (R) can be seen in the lumen. Bar: 5 μm.

Fig. 7. Fibroblasts (F) adjacent to longitudinal smooth muscle cells (L) in the portal vein from SHR. Bar: 10 μm.
in the paracellular connective tissue is relevant to ionic permeabilities, to contractility, and to pharmacomechanical coupling in normotensive versus hypertensive animals. Part of the reason for contradictory results may be due to age differences, and it is also possible that strain differences occur in different colonies. We found only smooth muscle cellular hypertrophy and no overall thickening of the longitudinal layer as has been previously reported for SHR (SUTTER and LJUNG, 1977; GREENBERG et al., 1978) and for stroke prone SHR (SHIMAMURA et al., 1989). Because of the major morphological alterations that may or may not occur due to age or colony, it would be most useful to have structural information along with functional studies to help clarify which characteristics are involved in those differences present in the portal vein from hypertensive animals.

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REFERENCES


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