Pressure-independent hypertrophy of veins and pulmonary arteries of spontaneously hypertensive rats. Characterization of function, structural and histochemical changes

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Summary
1. Portal veins, vena cavae and pulmonary arteries from spontaneously hypertensive (SH) rats were found to undergo medial smooth-muscle hypertrophy when compared with corresponding blood vessels from age- and sex-matched Wistar–Kyoto (WKy) normotensive rats. There was an increase in the density of the mucopolysaccharide and glycoprotein staining in veins, pulmonary arteries vena venorum and ventricular myocardium of SH rats.

2. Electron-microscopic examination of the blood vessels and myocardium of SH rats suggested enhanced protein synthesis and hypertrophy both in the smooth muscle and endothelial cells. Central venous, portal venous and right ventricular pressures were measured in SH rats and were not elevated when compared with corresponding values for WKy rats. Veins and pulmonary arteries from SH rats developed more tension when challenged with vasoconstrictor stimuli than corresponding vessels obtained from WKy rats.

3. Veins and pulmonary arteries obtained from SH rats demonstrated a greater uptake of $[^{14}C]$glycosamine than corresponding blood vessels from WKy rats.

4. These findings demonstrate the existence of pressure-independent hypertrophy of smooth muscle in blood vessels obtained from SH rats, associated with an increase in glycoprotein synthesis and an enhanced contractile activity of the muscle. The data suggest that the enhanced contractility of blood vessels from SH rats reflects the enhanced muscle mass. It may represent an aberrant humoral or cellular mechanism which results in the hypertension.

Key words: hypertension, muscle hypertrophy, pulmonary artery.

Abbreviations: SH, spontaneously hypertensive; WKy, Wistar–Kyoto.

Introduction
Cardiac and arterial smooth muscle hypertrophy are classical sequelae of experimental and human hypertension (Genest, Koiv & Kuchel, 1977). Volume or pressure overload in the heart, or increased intravascular pressure, appears to stimulate an increased synthesis of RNA and protein within the myocardial and vascular smooth muscle cells. This hypertrophy may be secondary to the increased afterload to the heart and to the raised intravascular pressure, respectively.

Folkow, Hallback, Lundgren, Sivertsson & Weiss (1973) have provided evidence which suggests that the increased vascular resistance of spontaneously hypertensive rats is mediated by an increase in the resistance vessel wall-to-lumen ratio. The mechanism of the smooth muscle hypertrophy and subsequent encroachment of the arterial media into the lumen which produced this change is unknown. Contraction of small arterioles (100 μm o.d.) may transmit an increased intravascular pressure to larger arterioles and arteries.
and stimulate smooth-muscle hypertrophy. Alternatively, an increased blood flow to vascular beds not requiring an increased delivery of oxygen may initiate the myogenic response of Bayliss contraction to vascular smooth muscle and increased intravascular pressure which then stimulates medial smooth-muscle hypertrophy. Both hypotheses, nevertheless, agree on the concept that the hypertrophic process results from increased intravascular pressure. However, were the hypertrophy of smooth muscle to occur in the absence of an increase of intravascular pressure, it is possible that the cellular changes initiating the smooth-muscle hypertrophy could be a causal abnormality in the development of hypertension.

Recent studies from our laboratory demonstrated that portal and femoral veins from spontaneously hypertensive rats were less extensible and demonstrated an enhanced contractility and synthesis of prostaglandins when compared with portal veins obtained from normotensive Wistar-Kyoto rats (Greenberg & Bohr, 1975; Greenberg & Curro, 1976, 1978; Greenberg & Wilborn, 1978). Since the veins were presumably not exposed to the increased intravascular pressure of hypertension it was assumed that the changes in extensibility, contractility and prostaglandin synthesis reflected intrinsic changes in the blood vessels and the smooth-muscle cells. The present study was therefore designed to evaluate the functional, structural and histochemical changes which occur in the veins and main pulmonary artery of spontaneously hypertensive rats.

**Methods**

Ninety male six-month-old Okamoto-Aoki, Wistar-Kyoto normotensive rats and spontaneously hypertensive rats were used in this study. Blood pressure was recorded in conscious animals with an automated tail-cuff plethysmograph (Marco Bio Systems, PE 300, Houston, TX). Heart rates were recorded on a Graph Model 5 Polygraph and read directly from the pulse tracing. After cervical dislocations portal veins from the factors of the splenic vein to their entrance into the liver lobe, were excised and prepared for recording of contractile tension as previously described (Greenberg, 1976). Main pulmonary arteries and vena cavae were removed, cut into rings, the rings opened along the circumference of the blood vessel, and the resulting strips of muscle were prepared for the recording of tension development as described below.

**Contractility studies**

Portal veins, pulmonary arteries (3 mm 0·0 × 1 mm in width) and vena cavae (3·5–3·8 mm 0·0 × 1 mm in width) were excised, placed into isotonic sodium chloride solution (154 mmol/l saline) at room temperature and, under a dissecting microscope, cleaned of adhering fat and fascia. Portal veins were cut in half longitudinally. The rings of pulmonary artery and vena cavae were cut open to provide a uniform muscle strip. The veins and artery were then mounted between a stationary glass rod and a Grass ft. 03C force-displacement transducer in a muscle bath containing saline at 37·5°C (pH 7·4). The contractile activity of the muscle was recorded on a Beckman Type R ink-writing oscillograph. The saline, aerated with a mixture of 95% O₂–5% CO₂, contained (mm): NaCl (128); KCl (4·9); CaCl₂ (1·6); MgCl₂ (1·2); NaHCO₃ (14·8); monosodium dihydrogen phosphate (1·18); dextrose (10); sucrose (50); and calcium disodium EDTA (0·026). Each vein and artery strip was stretched to the optimum portion of its length-tension curve as described previously (Greenberg, 1976). One portal vein, vena cava or pulmonary artery strip from both an SH rat and WKy rat was mounted in each of three muscle baths.

After a 60 min equilibration period, concentration–response curves were obtained to cumulative additions of various agonists, by the method of Van Rossum (Greenberg, 1976). Each muscle strip was exposed to six agonists; 60 min was allowed between the evaluation of the concentration–response curves for the different agonists. The order of agonist administration was varied in each experiment to account for any potential interaction between the agonists. The agonists employed were noreadrenaline, prostaglandin D₂, prostaglandin F₂α, 9α, 11α, epoxymethanoprostaglandin E₂; potassium chloride and calcium chloride. Data were expressed as a percentage of the maximal response for each agonist and as contractile tension. Tension was calculated from the following formula:

\[ T = \frac{f}{a} \]  

where:

\[ a = \frac{w}{1 \times d} \]  

Where \( T \) is tension in g/cm², \( f \) is the development of contractile force (g); \( a \) is the cross-sectional area of the vein or artery strip (cm²); \( l \) is the length
of the strip (cm) at the optimum portion of the length–tension curve; \(d\) is the density of the strip (1.05); \(w\) is the weight of the strip (g).

**Measurement of venous and right ventricular pressures**

In these experiments an anaesthetized in vivo preparation of SH and WKy rats was utilized for measurement of portal venous pressure, whereas conscious rats were employed for the measurement of the remaining pressures. Either anaesthetized rats were allowed to breathe spontaneously. The jugular vein was cannulated with a PE 20 cannula filled with heparinized saline (5 mg/l) which was then inserted into the inferior vena cavae. The neck wound was then sutured. A needle (21 gauge) connected to a cannula (PE 50) filled with heparinized saline was then inserted into the heart through the chest. Animals in which the left ventricle was punctured were discarded from the study. The SH and WKy rats were then allowed to recover and 15 min later venous pressure and right ventricular pressure were measured for 15 min with a Statham P23 Gb pressure transducer and the pressures recorded on a Beckman Type R dynograph. The animals were then killed by cervical dislocation.

In a separate series of experiments SH and WKy rats were anaesthetized with ether, a laparotomy performed and the portal veins cannulated before its entrance into the liver, the cannulae pointing toward the hindquarters. Pressure was recorded for 10 min, as described above, and the animals were then killed with an overdose of ether.

**Measurement of \([2^{14}C]\)glycosamine uptake into blood vessels**

Five SH and WKy rats were weighed and injected with 10 \(\mu\)Ci/kg 1.0 ml of \([2^{14}C]\)glycosamine (New England Nuclear, Waltham, MA; 25 \(\mu\)Ci/mg of glycosamine). Five SH and WKy rats were killed with an overdose of ether 22 h after the intraperitoneal injection. The portal vein, vena cavae and pulmonary artery were removed, and digested overnight with 0.1 ml of Biosolve (Amersham Searle, Chicago, IL). A portion of the solution was then assayed for protein content. The remainder of the solution was diluted with 10 ml of ScintiVerse (Fisher Scientific, Harrahan, LA) and the radioactivity counted in a Beckman LS scintillation counter. The counts were corrected for quenching and the efficiency of the counting (41-7%) by employing the method of external standards ratio. Data were expressed as d.p.m./mg of blood vessel protein.

**Light microscopy**

SH and WKy rats were killed by cervical dislocation, the portal vein, main pulmonary artery and the vena cava 1 cm below the level of the diaphragm were removed and fixed in 10% neutral buffered formalin containing 0.5% cetyl pyridinium chloride for the preservation of the water-soluble mucopolysaccharides. After 12 h fixation, the tissues were embedded in paraffin, serially sectioned into 6 \(\mu\)m slices and subsequently mounted on chemically clean slides. Alternate sections were stained with haematoxylin and eosin, periodic acid–Schiff stain for neutral carbohydrates and acid glycosaminoglycans, Alcian Blue at pH 2.5 for non-sulphated sialoglycoproteins and Alcian Blue at pH 0.5 for sulphated glycoproteins. Sections from the blood vessels obtained from the hypertensive and normotensive rats were stained simultaneously in the same bath to eliminate any temporal variability due to different batches of stain.

**Electron microscopy of blood vessels from SH and WKy rats**

Portal veins, pulmonary arteries and vena cavae were removed from five SH rats and five WKy rats, fixed for 4 h in 2% glutaraldehyde in 0-075% cacodylate buffer (pH 7.4) containing 4.5% sucrose, 1.2 mm-CaCl\(_2\) and 0.25% cetyl pyridinium chloride to enhance preservation of the glycoproteins. After the vessels were fixed and hardened they were placed in fresh cacodylate buffer for 4 h, washed in the same buffer and post-fixed for 2 h in 2% osmium tetroxide at room temperature. The tissues were then dehydrated with ethanol and embedded in Spurr's resin.

Sections (1 \(\mu\)m thick) were then cut, placed on glass slides, stained with Toluidine Blue, and examined with the light microscope to determine the best orientation for ultramicrotomy. Serial ultrathin sections were then cut with an LKB Ultramicrotome, placed on grids to allow identification of serial sections for three dimensional reconstruction of the components of the vascular wall, stained with lead citrate and uranyl acetate, and the sections examined in a Phillips EM 30 transmission electron microscope. Electron micrographs were taken at the same magnification of
corresponding regions from blood vessels obtained from WKy and SH rats.

**Results**

**Haemodynamics**

Systolic arterial pressure was elevated in SH rats (183 ± 3) compared with ten WKy rats (117 ± 4). Central venous, portal venous and right ventricular pressures were similar in WKy and SH rats ($P > 0.05$).

**Contractility**

Portal veins from SH rats developed more tension to noradrenaline (356 ± 65 g/cm²) when compared with the responses of portal veins from WKy rats (172 ± 39) ($n = 5, P > 0.05$). Similar findings were obtained in vena cavae (SH rats, 241
Vascular hypertrophy in SH rats

**Table 1. Biochemical values from vessels of normotensive (WKY) and hypertensive (SH) rats**

<table>
<thead>
<tr>
<th></th>
<th>Portal vein</th>
<th>Inferior vena cavae</th>
<th>Pulmonary artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SH</td>
<td></td>
</tr>
<tr>
<td>Weight (mg/dry wt.)</td>
<td>3.1±0.2*</td>
<td>4.2±0.3*</td>
<td></td>
</tr>
<tr>
<td>Protein (µg/mg)</td>
<td>231±24</td>
<td>394±47*</td>
<td></td>
</tr>
<tr>
<td>[14C]Gluosamine</td>
<td>27±4</td>
<td>42±9*</td>
<td></td>
</tr>
<tr>
<td>(10^{-3} x d.p.m./mg of protein)</td>
<td>2±0.4</td>
<td>1.9±0.5</td>
<td></td>
</tr>
<tr>
<td>[14C]Thymidine</td>
<td>231±24</td>
<td>394±47*</td>
<td></td>
</tr>
<tr>
<td>(10^{-3} x d.p.m./mg of protein)</td>
<td>27±4</td>
<td>42±9*</td>
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Contractile tension was greater in blood vessels from the lesser circulation of SH rats when prostaglandins, serotonin, KCl, angiotensin II and bradykinin were employed as agonists.

Light microscopy

Pulmonary arteries, inferior vena cavae and portal veins of SH rats were found to have thicker walls and more smooth muscle in comparison with corresponding vessels of normotensive WKY rats (Fig. 1). The ability of the vessels from SH rats to take up periodic acid-Schiff stain, indicative of increased mucopolysaccharide synthesis, was greater than that in the vessels obtained from WKY rats. Electron microscopic studies demonstrated a hypertrophic smooth-muscle cell approximately twice the diameter of that found in the veins of the WKY rats. Associated with the hypertrophy was an increase in the size and number of Golgi organelles indicative of increased protein synthesis. In addition, disproportionately enlarged nucleoli in nuclei of hypertrophic veins and pulmonary artery from SH rats indicated that DNA-directed RNA synthesis was enhanced in the low pressure vessels of SH rats (Greenberg, Palmer & Wilborn, 1978).

Biochemical properties of veins from SH rats

[14C]Gluosamine, a precursor for membrane and cellular glycoproteins, was found to be accumulated to a greater degree in portal veins, vena cavae and pulmonary arteries obtained from SH rats when compared with WKY rats (Table 1). [14C]Thymidine, an index of DNA-synthesis was not significantly different in veins and pulmonary artery obtained from SH rats when compared with WKY rats (Table 1). Previous studies demonstrated an increase in prostaglandin synthesis and a decrease in cyclic AMP in veins obtained from the SH when compared with WKY rats (Greenberg, 1976; Greenberg et al., 1977; Greenberg & Wilborn, 1978). The protein content/g wet wt. was also greater in veins and pulmonary artery obtained from SH when compared with WKY rats (Table 1).

Discussion

Results from this study and those published elsewhere demonstrate that vena cavae, portal veins and pulmonary arteries obtained from SH rats demonstrate hypertrophy in the absence of an increase in corresponding vascular pressures (Greenberg & Curro, 1978; Greenberg & Wilborn, 1978; Greenberg et al., 1978; Wilborn, Greenberg & Palmer, 1978). The hypertrophy is not the result of fixation artifact or contracture of muscle fibres since: (1) the muscles were fixed in a solution containing nitroglycerine to relax the muscles; (2) the hypertrophy is accompanied by an increase in the protein content of the blood vessels obtained from the SH rats; and (3) the hypertrophy can be selectively reversed by antihypertensive drugs (Greenberg et al., 1978). These results clearly suggest that vascular smooth-muscle hypertrophy can occur in the SH rats in the absence of an increase in intravenous and intrapulmonary artery pressures. It is possible that the increased arterial pressure may liberate a stimulator of cell growth or a derepressor of an inhibitor of cell growth from the kidney or arterial side of the circulation. A study by Crane (1962) demonstrated that unilateral nephrectomy resulted in a 28% decrease in the incorporation of 35-S-SO4 into sulphate mucopolysaccharides of mesenteric artery obtained from normotensive rats, suggesting renal control of vascular smooth-muscle mucopolysaccharide synthesis. Alternatively, aberrant changes within the
vascular smooth muscle cell may result in hypertrophy. DNA-directed RNA synthesis may be inhibited by a process which is under the control of cyclic AMP. Enhanced synthesis of prostaglandins may result in a diminution of cyclic AMP synthesis, removal of the inhibitory effect on cell growth and enhanced cellular synthesis of proteins and mucopolysaccharides, such as glucosamine-containing glycosaminoglycans.

Previous studies from our laboratory (Greenberg & Bohr, 1975; Greenberg, 1975) demonstrated an enhanced venous contractility in portal veins obtained from SH when compared with WKy rats. Although a multitude of defects are evident in the veins of SH rats a direct correlation between them and the enhanced contractility was not forthcoming. The results of the present experiments provide a theoretical, and perhaps realistic framework, to explain the enhanced contractility.

The contractility of vascular smooth muscle related to the ATP-splitting activity of the actomyosin rather than the actual actomyosin content of the muscle. If the contractile proteins of the hypertrophied venous smooth muscle demonstrate more ATPase activity in SH compared with WKy rats, or if the contractile proteins are somewhat different, then the hypertrophic smooth muscle and the associated changes in the contractile proteins explain the increased contractility of veins obtained from the SH rats. Furthermore, the demonstration of pressure-independent hypertrophy indirectly suggests the possibility of arterial smooth-muscle hypertrophy initiating hypertension.

Acknowledgments

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References


