Ploidy in Mesenteric Vessels of Aged Spontaneously Hypertensive and Wistar-Kyoto Rats

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Long-term regulation of blood pressure in a hypertensive rat may be mediated by elevated DNA content of smooth muscle cells of resistance vessels. This study explores DNA changes represented by an increased frequency of polyploid cells in multiple levels of the mesenteric arterial tree of spontaneously hypertensive rats (SHR) and age-matched Wistar-Kyoto (WKY) control rats. Two ages were examined: 45 and 78-80 weeks of age. SHR and WKY rats did not differ in frequency of polyploid cells at any mesenteric branch level at either age. Although hypertension per se seemed not to be a factor, both species showed increased numbers of polyploid cells with aging at certain branch levels of the mesenteric arterial tree. The data in the current study support the idea that hypertension and aging may result in similar and possibly additive changes in DNA in the vessel wall. (Hypertension 1989; 13:475-479)

We have suggested that the elevation of DNA content in vascular smooth muscle of hypertensive rats might have long-term effects on the regulation of blood pressure. This hypothesis grew out of our observation of increased DNA content, that is, hyperploidy, of smooth muscle in the aortas of spontaneously hypertensive (SHR) and Goldblatt rats. Folkow and others have previously inferred that structural changes leading to decreased luminal diameter of resistance vessels are central to the increased peripheral resistance common to most forms of chronic hypertension. Cowley et al have also argued that increased peripheral resistance represents a common end-stage picture masking the primary etiology of many forms of hypertension. Increased wall thickness associated with increased DNA content would be particularly interesting for two reasons: First, we now have more knowledge of the molecular mechanisms controlling smooth muscle replication than we do about the controls over smooth muscle mass. Second, the irreversibility of increases in DNA might imply a fixed increase in the ability of the hypertensive vessel wall to synthesize proteins and maintain vascular mass. Those changes might themselves then contribute to the maintenance of structural changes in the vessel wall.

While our data on hyperploidy were confined to the aorta, a vessel not involved in control of resistance, there is clear evidence that DNA replication also occurs in smooth muscle of the hypertensive microvasculature, although the increase in DNA is apparently due to an increase in cell number rather than in DNA content per nucleus. Ploidy analyses of microvessels failed to show any difference between 3.5-month-old SHR and age-matched Wistar-Kyoto (WKY) rats. Morphometric studies, however, have shown that smooth muscle cell number is increased in mesenteric arteries of hypertensive rats.

Thus there seem to be two forms of DNA replicative responses in hypertension: hyperplasia in small vessels and hyperploidy in large vessels. This conclusion is complicated by two additional observations. First, we know that the frequency of polyploid aortic smooth muscle cells in hypertensive rats increases with age. It is possible that the frequency of polyploidy in hypertensive microvessels increases at a different rate than the frequency in larger vessels. Second, normotensive animals, including rats and humans, show increases in the proportion of polyploid aortic smooth muscle cells in large vessels as a function of age. This is of interest because many arterial morphological changes associated with aging are also seen in vessels from chronically hypertensive animals or atherosclerotic vessels and, in fact, may be exacerbated by hypertension superimposed on the aging process. The present study extends this correlation to show that ploidy increases...
with age in the microvascular smooth muscle cell, although the change in the microvasculature is independent of hypertension.

**Materials and Methods**

Male SHR and WKY rats from Charles River Laboratories (Wilmington, Massachusetts) arrived at age 13 weeks, were housed in our facility and given rat chow and water ad libitum. Mean arterial blood pressures were measured at random intervals using the tail-cuff method with a plethysmograph (Narco Biosystems, Houston, Texas). Rats were warmed with a heat lamp for 5 minutes before attempting measurements. At least three readings were averaged to give a measurement for the day. At either 45 weeks of age or 78–80 weeks of age, rats were killed by ether overdose. Aorta and small intestine with associated mesentery were removed and placed into Waymouth’s tissue culture medium buffered with 0.01 M HEPES.

Medial preparations of aortas were minced and digested in a collagenase (170 units/ml) and elastase (15 units/ml) mixture in buffered Waymouth’s media.3 With incubation at 37° C and periodic pipetting, individual smooth muscle cells dissociated from the tissue. Suspensions were filtered through 70-μm wire mesh to remove large, undigested pieces and were centrifuged at 460g for 7 minutes. The cells were then suspended in 10 ml phosphate-buffered saline (PBS), centrifuged, and resuspended in 1 ml PBS to which 3 ml 100% methanol was added slowly. After centrifugation, the pellet was suspended in 100% methanol, and fixed cells were dropped onto slides for subsequent Feulgen staining.

A loop of ileum and mesentery was pinned out, flushed with cold Ringer’s lactate via the superior mesenteric artery, and five branch levels of the mesenteric arterial tree were dissected. Type I vessels were defined as those entering the gut, with types II, III, and IV being vessels of increasing size (see Figure 1). Type IV vessels branched from the superior mesenteric artery. Although there was some variation from arcade to arcade, only those vessels that could be identified without confusion were taken. Three to six vessels of each of types II, III, and IV were taken from each rat. Because of the small size of type I vessels, 10–15 were taken for dispersion.

For each rat, vessels from individual branch levels were pooled, and vessels were partially digested in collagenase and elastase at 37° C. After approximately 2 hours in enzyme, cells were still loosely associated in a “vessel” structure and were transferred to a slide where the vessel was mechanically disrupted with forceps to further isolate cells. After drying overnight, cells were fixed in 100% methanol for 30 minutes. Because the cells were still in a vessel-like structure on the slide, when dried the vessel collapsed on itself. Cells in the center overlapped and were unreadable for DNA content. We believed endothelial cell contamination would be minimal due to this “enforced” avoidance of luminal cells.

Both aortic and mesenteric arterial cells were Feulgen-stained by a modified method of Fand.22 Optical density per cell was read on a digital microdensitometer image analysis system23 with a 546±10 nm interference filter, and the percentages of total cells that were diploid (2N) or tetraploid (4N) and above were determined. At least 200 cell nuclei were analyzed for each branch level per rat. Unpublished data from this laboratory show similar results of DNA measurements by three different techniques: Feulgen microdensitometry, flow cytometry, and propidium iodide/photomultiplier-tube-based fluorimetry. Because of small numbers of cells, we chose to use the Feulgen method on single cells.

Percentages of 4N cells at each arterial branch level were compared by two-way analysis of variance (ANOVA)24 to look for differences due to age or strain of rat. An α level of 0.05 was accepted as significant, although a conservative interpretation must be used when several ANOVA’s are run due to random chance that one test may show insignificance. Since the data for older SHR superior mesenteric arteries were lacking, significance within this arterial group was tested using Student’s t test.

**Results**

As seen in Table 1, blood pressures were different between conscious, restrained SHR and WKY rats at both ages reported here. SHR blood pressure was consistently 45–55 mm Hg higher than that of comparably aged WKY rats. Blood pressures previously reported for year-old WKY rats have ranged from 89 mm Hg in etherized rats3 to 135 mm Hg.12 In our study, 45-week-old WKY rats exhibited a mean
neither age nor blood pressure affects the smooth muscle cells of the aorta was an increase of both increasing age and hypertension (p<0.005 for each, two-way analysis of variance [ANOVA]). In smaller arteries of the mesenteric tree, no difference was shown between hypertensive and normotensive rats. Numbers of polyploid smooth muscle cells did increase as a function of age in type IV (p<0.025), III (p<0.005), and II (p<0.01) arteries as determined by two-way ANOVA. Neither type I vessels nor superior mesenteric arteries showed any significant differences with age or strain. Numbers represented are mean±SEM.

Discussion

These data in aging rats confirm our earlier study done in 3.5-month-old rats showing that there is no difference in frequency of polyploid cells from mesenteric microvessels of hypertensive and normotensive rats.8 There is, however, an increase in both strains as a function of age and the location of the vessel within the microvasculature. It remains possible that microvascular smooth muscle cells undergo an accelerated rate of formation of polyploid nuclei during hypertension. Data by Black et al25 do not support this idea. They reported ploidy in mesenteric arteries of both SHR and WKY rats at four different ages: 12, 26, 32, and 40 weeks of age. In their study, they saw no difference with increasing age or hypertension in the frequency of polyploid cells in the mesenteric arteries. If we pool our data from types I to IV mesenteric arteries to approximate their data from pooled tissues, the numbers are consistent at 40 and 45 weeks of age. An inconsistency exists, however, between their data and that of Owens et al10 in ploidy frequencies in mesenteric vessels from younger animals (12 weeks for Black et al25 vs. 15 for Owens et al10). Owens et al10 report percentages of 2.0–4.0 (average 2.7) for SHR, lower than the 6.1% reported by Black et al25 vs. 15 for Owens et al10. Enough variability seems to exist among methods,
investigators, and perhaps rats to make a comparison of data difficult.

It is interesting that the type I vessels maintain low levels of polyploid smooth muscle cells in both strains of rats examined here. Smooth muscle cells comprising the larger vessels may be under different demands for active constriction than smooth muscle in the type I vessels, those closest to the gut, where blood pressure has already fallen. Bohlen et al. report that pressures in both 100 and 50 \( \mu \)m vessels in rat cremasteric muscle are approximately half of the systemic blood pressure in 7–8-week-old SHR. Type I mesenteric vessels from 3-month-old SHR are approximately 75 \( \mu \)m in diameter. Thus, although Bohlen et al. report microvascular pressures that are higher in SHR than normotensive rats, the smallest vessels studied here from either SHR or WKY rats may not be exposed to an increase in pressure great enough to stimulate a response.

The hypothesis that polyploidy can represent an adaptation to pressure demands fails to explain the apparent lack of occurrence of polyploidy in the smooth muscle cells of the superior mesenteric artery of SHR. Lee et al. report that, in 10–12-week-old SHR, the smaller muscular arteries and arteriolar vessels of the mesentery were increased in medial thickness, although superior mesenteric arteries had not become thickened at this age. By 28 weeks, however, the mesenteric arteries do exhibit medial thickening, presumably due to increases in intercellular space and probable hypertrophy of the smooth muscle cells. This same response was seen in Dahl salt-sensitive rats as well. The absence of a difference between hypertensive and control rats in the number of smooth muscle cell layers was taken as an indication of lack of hyperplasia. Without data on thymidine incorporation, however, the possibility exists that an increase in DNA content of the superior mesenteric artery may still be occurring as an increase in ploidy rather than as a result of cell replication and division. Our data suggest that this is unlikely. Another possibility, of course, is that there is a minimal, undetectable increase in DNA content of the superior mesenteric artery with either age or hypertension.

Black et al. show an increase over time in polyploidy in both strains. Polyploidy in WKY rats does not increase dramatically up to 40 weeks of age; neither was there a significant increase in the present study between 45 and 78 weeks of age (6.5 to 7.0%). In the study by Black et al., SHR exhibited a greater increase over time in ploidy in superior mesenteric artery than did WKY rats. At 40 weeks of age, the SHR showed high levels of polyploidy, from 14 to about 20%. The 45-week-old SHR reported here showed only a 7.6% complement of 4N smooth muscle cells. We find this discrepancy difficult to understand unless it is the result of variability among rats due to source or sex differences.

We also want to point out that the increase in polyploidy seen here and the microvessel hyperplasia reported by others are not necessarily mutually exclusive. Both polyploidy and increase in cell number could exist in the same vessel, resulting in even greater increases in total DNA content. Furthermore, the absence of ploidy differences in microvessels of SHR and WKY rats observed in the present study does not exclude the possibility that cell enlargement or hypertrophy might contribute to changes in vessel mass. In previous studies of smooth muscle cell hypertrophy in large vessels of SHR and Goldblatt hypertensive rats, we found that smooth muscle cells from each respective ploidy class in hypertensive rats were hypertrophied relative to the corresponding cells from normotensive control rats.

Changes in DNA content of the vessel wall, whether by the process of hyperplasia or an increase in ploidy to a 4N or greater complement of DNA per cell without a decrease in cell number, represent a form of fixed structural change not easily reversible without cell death. Warshaw et al. treated SHR and WKY rats with hydrochlorothiazide, hydralazine, and reserpine and found a decrease in smooth muscle cell number and size in both strains. Change in cell number must occur by cell death or cell fusion, presumably the former, although Warshaw et al. did not report on DNA content of the vessels. In contrast, Owens has reported that, although antihypertensive treatment can prevent increases in DNA if applied to young SHR and WKY rats, increased DNA content is maintained in rats treated after establishment of hypertension. Likewise, Lichtenstein et al. have reported that reversal of hypertension failed to decrease frequency of tetraploid aortic smooth muscle cells. An excess DNA load in resistance vessels may predispose the vessels to hyperreactivity in response to subsequent hypertrophic stimuli.

Finally, Haudenschild and Chobanian reported that antihypertensive therapy may reduce aging effects on the vasculature. Our data, showing that an increase in frequency of polyploid smooth muscle cells occurs in larger mesenteric arteries in both SHR and WKY rats with age, also support the idea that similar vascular changes may occur in hypertensive and aged animals.

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References


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