Lysosomes in Aortic Smooth Muscle Cells

Effects of Hypertension

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Hypertension induces hypertrophy and increased turnover of aortic smooth muscle cells along with an accumulation of connective tissue in the aortic wall. We identified the lysosomes in normal and hypertensive aortic muscle cells by light and electron microscopy, utilizing cytochemical staining for acid phosphatase activity. Lysosomes were found to be more numerous in hypertensive vessels. Biochemical assays of two specific lysosomal enzymes revealed a doubling of acid phosphatase and a more than threefold increase in β-*N*-acetyl-glucosaminidase activities in hypertensive aortas (Am J Pathol 73:727–734, 1973).

THE PRESENCE OF MANY DENSE BODIES in activated vascular smooth muscle cells [1–3] and the demonstrated association of a lysosomal response with tissue damage [4,5] suggests that lysosomes are involved in vascular injury. Peters, Muller and deDuve have recently isolated a particulate fraction enriched in lysosomal hydrolases from the aortic wall, but attempts to identify lysosomes cytochemically and ultrastructurally have yielded equivocal results.[6]

Experimental hypertension is an excellent model of vascular injury. This type of stress causes degeneration,[3,7] cellular hypertrophy [8,9] and increased turnover,[10] together with accelerated synthesis of fibrous proteins [9] and mucopolysaccharides.[11] This setting appeared ideal to demonstrate by ultrastructural cytochemistry the presence of lysosomes in vascular smooth muscle cells and to study their role in vascular injury and repair.

Materials and Methods

Ten young male Carworth (CF-N) rats with renal clip hypertension (greater than 150 mmHg systolic pressure) and a similar number of normotensive controls were maintained for 8 weeks; final body weights were similar in both groups. Tho-

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racic aortic segments delimited by the left subclavian and coelic arteries were then removed.[9] Enzymes and DNA determinations were carried out on intima-media layers of pairs of thoracic segments after their separation from adventitia;[12] four sets of normotensive pairs and four sets of hypertensive pairs were analyzed. Intima-media fragments from each pair were placed in distilled water and homogenized for 6 minutes with a Teflon® pestle and mortar; temperature was maintained at 4 C throughout. Centrifugation at 4 C at 1000*g* for 10 minutes was followed by separation of the supernatant for enzyme analyses; the pellet was used for DNA determinations. Completeness of this separation was confirmed by analyses of pellets for enzyme activity and of supernatants for DNA. Total DNA content per aortic pair was determined by the method of Ceriotti [13] modified by Hubbard *et al.*[14] Total β-*N*-acetyl-glucosaminidase activity was assayed using the appropriate *p*-nitrophenyl substrate and the method outlined by Barrett [15] after appropriate reduction of volumes of reagents. Acid phosphatase activity was assayed using β-glycerophosphate as the substrate and following the method of Barrett,[15] again modified for the small amount of tissue. Time curves were run to determine optimal conditions and appropriate reagent, and tissue blanks were checked for baseline activity. Enzyme activities reported are based on μmoles of nitrophenol [16] released by the β-*N*-acetyl-glucosaminidase and μmoles of inorganic phosphorus released by the phosphatase, using the modified method of Fiske and Subbarow.[17] Activity is expressed in terms of total μmoles of each enzyme released per hour per two aortas.

Histochemical studies were carried out on rings of thoracic aortas and aortic arches from 4 hypertensive and from 4 normotensive animals. Thin (approximately 2 mm) slices of vessel were fixed for 2½ hours at 4 C in 3% glutaraldehyde (Polysciences, Inc, Warrington, Pa) in 0.1 M cacodylate buffer, pH 7.4.[18] Freely floating frozen sections (10 μ for light microscopy, 30 μ for electron microscopy) were incubated for 20 minutes (electron microscopy) and 45 minutes (light microscopy) in a modified Gomori acid phosphatase medium with β-glycerophosphate as substrate.[19] For light microscopy the sections were treated with dilute ammonium sulfide, rinsed and mounted on slides with a water-soluble medium. For electron microscopy, incubated sections were rinsed, postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for 1 hour, soaked in 0.5% uranyl acetate in veronal acetate buffer at pH 5 for 1 hour, dehydrated and embedded in Epon 812. For routine ultrastructural studies, samples fixed in glutaraldehyde were directly postfixed in osmium as above. All substrates for cytochemical and biochemical studies were obtained from Sigma Chemical Company, St. Louis, Mo.

Results

Light microscopic histochemical studies demonstrated stained cytoplasmic granules (lysosomes) with acid phosphatase activity concentrated in the perinuclear area of smooth cells of normotensive aortas; the structures were generally quite small, up to approximately 0.5 μ in size (Figure 1A). In hypertensive animals, lysosomes were clearly increased in number and showed a wider variation of size than in normotensives (Figure 1B). In the electron microscope, several types of cytoplasmic structures, delimited by a single unit membrane, appeared to be likely candidates for identification as lysosomes. These included dense bodies (Figure 1C), autophagic vacuoles containing partially degraded

cytoplasmic structures such as mitochondria and rough surfaced endoplasmic reticulum (Figure 1D), and multivesicular bodies. In tissues incubated for acid phosphatase activity, reaction product was seen on dense bodies and autophagic vacuoles, identifying these bodies as lysosomes; multivesicular bodies were unreactive. In cells of normotensives, reactive lysosomes were less numerous and were usually single, scattered bodies; in hypertensives they were more numerous and were often in clusters (Figure 1E).

Results of enzyme analyses and DNA contents are shown in Table 1. Acid phosphatase activity was doubled and *N*-acetyl-glucosaminidase activity was increased more than threefold in hypertensive aortas, whether activities were expressed as totals or based on amounts of DNA. Total DNA was not different in the two groups. This indicates that increased activities in hypertensive vessels are not related to changes in cell numbers in these vessels.

Discussion

Our findings, using specific subtrates for two lysosomal enzymes, demonstrate a distinct lysosomal response in hypertensive vascular disease. It is of interest that similar elevations of lysosomes could be shown cytochemically and by assay as early as 3 weeks after the onset of hypertension.[20] Previous studies of the effects of hypertension on vascular enzymes by Albrecht[21] and Zemplenyi[22] showed increased acid phosphatase activity in the hypertensive rat aorta. However, these studies utilized diphenyl phosphate and *p*-nitrophenylphosphate substrates, which Beaufay has clearly shown are not specific and are not suitable indicators of lysosomal acid phosphatase activity.[23]

Table 1—Aortic DNA and Lysosomal Enzymes

	DNA*	Acid phosphatase†	N-acetyl-glucosaminidase†
Normotensive			
1	35.4	1.266	1.584
2	35.9	1.170	1.512
3	39.3	0.938	0.936
4	35.0	0.911	1.260
Hypertensive			
1	31.9	2.118	5.292
2	41.9	2.732	5.643
3	36.3	1.852	4.037
4	33.3	1.899	4.266

* μg/aortic pair
† μmoles substrate released/aortic pair/hr

It is not clear from these studies whether the lysosomal changes reflect cell damage, attempted repair, increased cellular uptake of phasma constituents or a combination of these events. The enhanced vascular permeability which occurs with hypertension [24,25] would cause increased exposure of mural cells to circulating plasma elements and increased endocytosis. It is also possible that cell degeneration resulting from the above or from increased wall tension could lead to release of lysosomal hydrolases and perpetuate a low-grade injury beyond the time of exposure to the initial stimulus. Study of the cellular uptake of parenterally administered tracers should make it possible to distinguish the effects of permeability changes from those due to mechanical damage by analysis of rates of uptake and gradients of lysosomal activity in the vessel wall. Others have found increases of aortic lysosomal enzymes in areas involved with atherosclerotic lesions in humans [26,27] and in experimental animals.[28] The possible associations of these enzymes with features of atherosclerosis largely parallel those mentioned above for hypertension, a proven accelerator of atherogenesis. These findings suggest that lysosomes may be a common mediator of cell damage in different forms of vascular disease.

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[*Illustrations follow*]

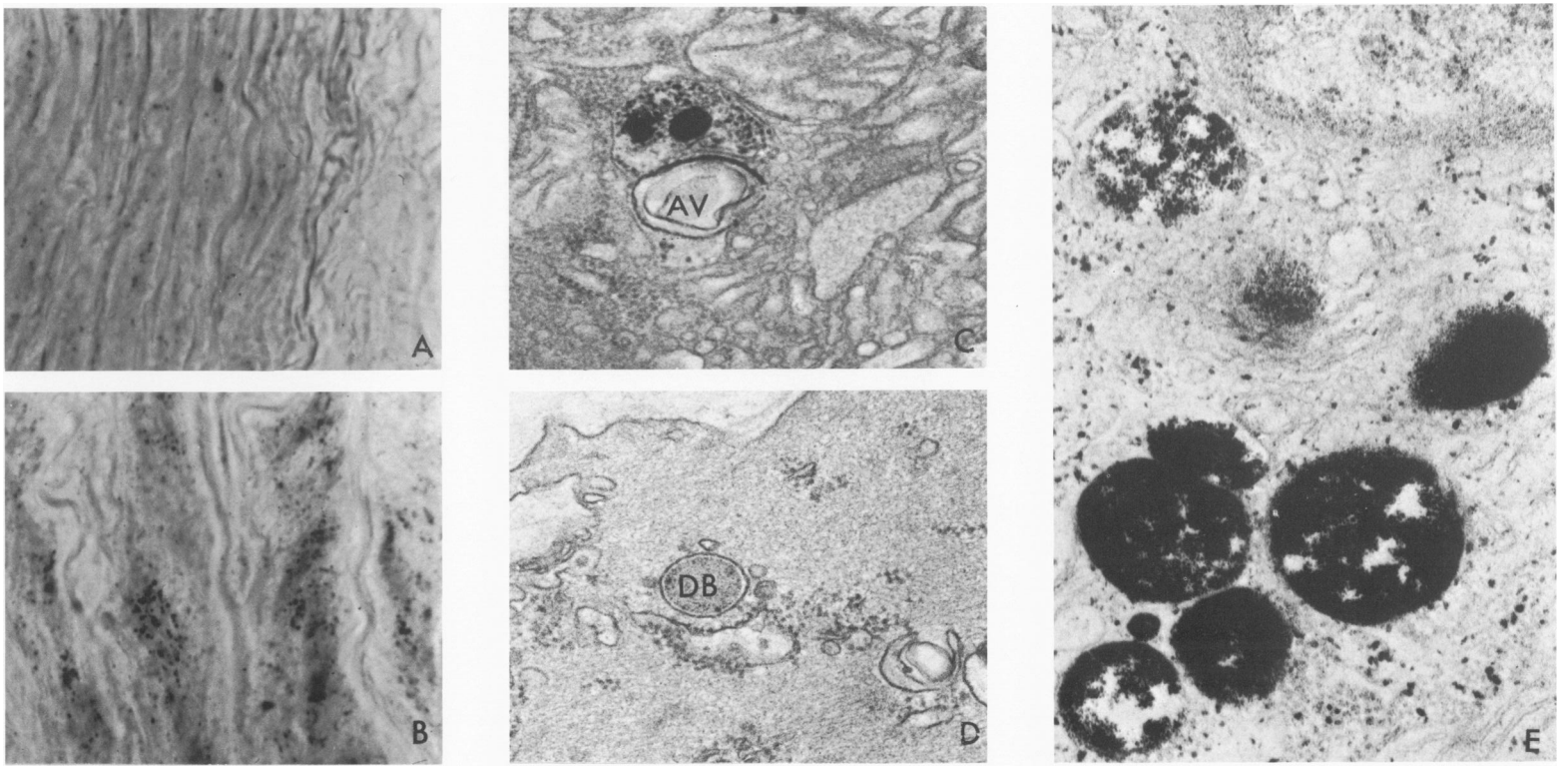

Fig 1—Rat aortas. **A** and **B**—Light micrographs of frozen sections of normal (**A**) and hypertensive (**B**) vessels incubated for 30 minutes for acid phosphatase activity. Lysosomes are more abundant and darkly stained in the hypertensive vessel. **C** and **D**—Electron micrographs illustrating two types of lysosomes: an autophagic vacuole (*AV*) and a dense body (*DB*). **E**—Electron micrograph of a frozen section of a hypertensive aorta incubated 20 minutes for acid phosphatase activity. Clusters of reactive lysosomes are identified in the cytoplasm of smooth muscle cells (**A** and **B**, × 650; **C**, × 48,000; **D**, × 50,000; **E**, × 48,000).

[*End of Article*]