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Research Articles

Elastic arteries in a primitive vertebrate: mechanics of the lamprey ventral aorta

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Abstract. Lampreys exhibit important structural and biochemical differences in their connective tissues relative to higher vertebrates. The lamprey ventral aorta wall, for example, is composed principally of microfibrils, not elastin and collagen as in higher vertebrates. Our working hypothesis was that this arrangement of microfibrils is primitive, but provides sufficient elastic behaviour to function as aortae in higher vertebrates. To support this hypothesis, we measured the mechanical properties of the ventral aorta wall of the lamprey, and showed that the architecture provides a mechanical structure that does produce functional mechanical properties similar to aortae in higher vertebrates.
Key words. Artery; lamprey; microfibrils; aorta; elastin; biomaterials.

Lampreys are one of the most primitive living vertebrates, thus studies of their tissue structure and function are of phylogenetic interest. Structural and biochemical differences have been identified between the connective tissues of lampreys and higher vertebrates. A good example of this is the lamprey aorta wall, which has no elastin-containing fibres or laminae and less collagen than that of higher vertebrates[1]. Elastin has not been found anywhere in lampreys, although the protein has been identified in all other vertebrates examined[2]. Microfibrils, which are the major component of the lamprey aorta wall, may produce a wall architecture that can function mechanically as an elastic reservoir. We demonstrate here that the ventral aortae of lampreys exhibit functional mechanical properties that are similar to those found in higher vertebrates. We believe that this is the first study to quantify mechanical behaviour of a microfibril-based elastic material, and as such may enhance our understanding of the evolution of structure and function in connective tissues. A microfibril-based elastic material may also provide functionally important mechanical properties for the suspensory ligaments (ciliary zonule) supporting the lens of mammalian eyes[1].

Among vertebrates the composition of the lamprey aorta wall is distinct. In most higher vertebrates (fig. 1A, B) the tunica intima consists of an endothelium and a subendothelial layer of a few fibroblasts, smooth muscle cells, collagen and longitudinally oriented elastin fibres. The tunica media is composed of alternating concentrically arranged, fenestrated elastin laminae (sheets), and smooth muscle cells that are surrounded by collagen, and finally, an adventitia of longitudinally arranged collagen fibres intermixed with elastin fibres and fibroblasts. In lamprey (fig. 1C, D) the tunica intima consists of a layer of endothelium similar to that lining the blood vessels of most vertebrates. The thick tunica media is composed of alternating concentric layers of smooth muscle cells and a dense fibrous extracellular matrix composed predominantly of tubular microfibrils 11–17 nm in diameter. The majority of these microfibrils are oriented parallel to the long axes of the adjacent smooth muscle cells. The smooth muscle cells have two orientations in the media; directly beneath the endothelium the smooth muscles are arranged longitudinally in a thin layer, while in the rest of the media the smooth muscle cells are arranged circumferentially. Scattered throughout the media between the microfibrils and the smooth muscle cells are small bundles of collagen fibrils. The outermost adventitia consists of collagen fibrils, some microfibrils and fibroblasts.

The composite construction of the vertebrate aortic wall provides a functionally important elastic behaviour; the wall is easily deformed under low pressures, but becomes very stiff at high pressures. Both elastin and collagen contribute to the development of this behaviour, although the contributions are functionally distinct. Elastin is a highly extensible, rubber-like protein that deforms when the artery is subjected to relatively low pressures, thus the low stiffness region of the stress-strain curve typically found in this pressure regime can be attributed to the mechanical properties of elastin. Collagen is about 1000 times stiffer than elastin, and is arranged in the arterial wall so that it is deformed only when the artery is subjected to high pressure, thus the stress-strain curve in the high pressure regime is characterized by high stiffness. This elastic behaviour is

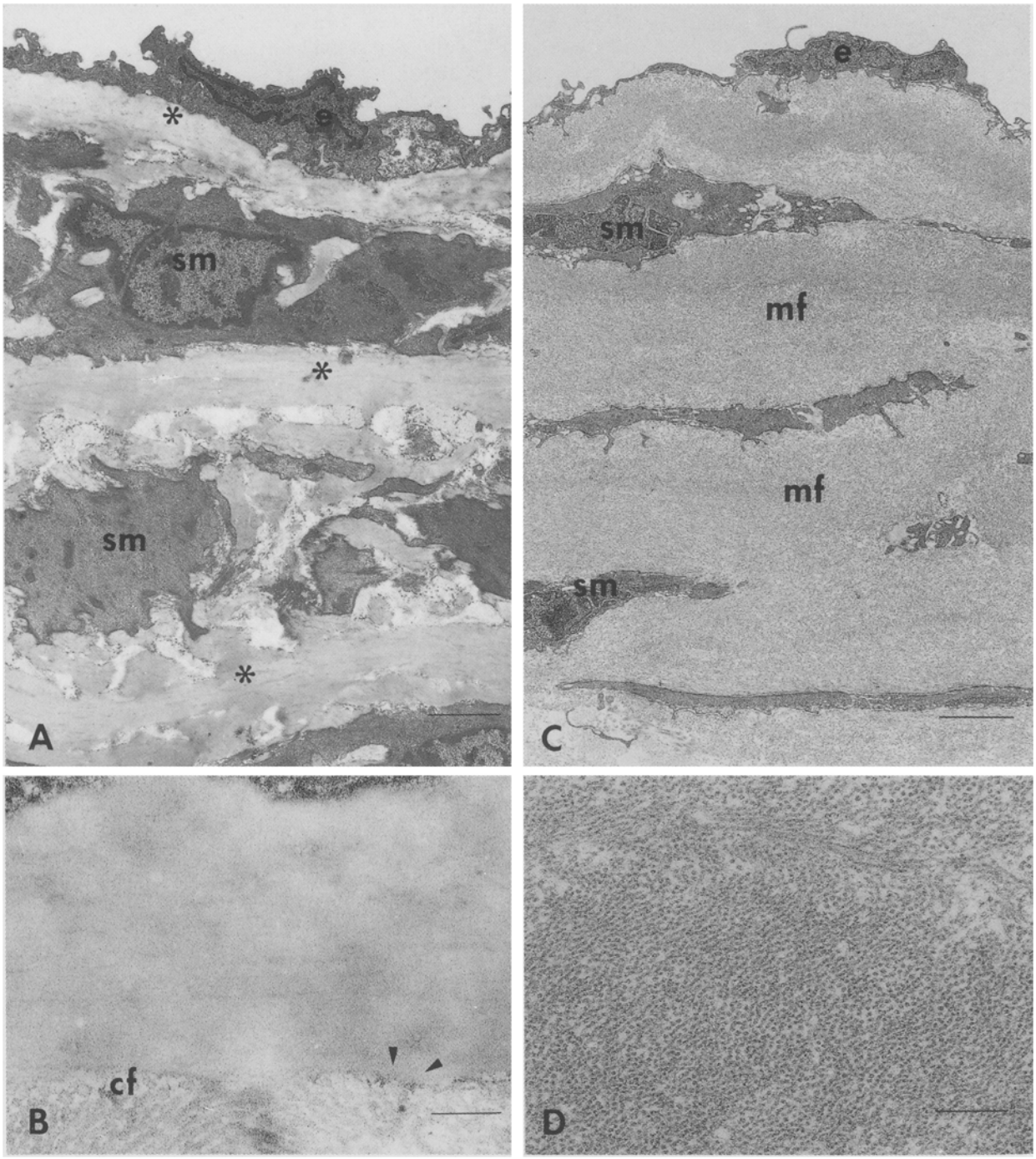

Figure 1. *A* Electron micrograph of a portion of the aorta from a rat showing endothelium (e), smooth muscle (sm) and a connective tissue consisting of large elastic fibres (*). *B* Higher magnification of the elastic fibre, note that it consists almost entirely of amorphous elastin. A few collagen fibrils (cf) and microfibrils (arrowheads) are also present. *C* Corresponding micrograph of lamprey ventral aorta showing endothelium (e), smooth muscle (sm) with connective tissue consisting predominantly of microfibrils (mf). Note the absence of amorphous elastin. *D* Higher magnification reveals the small diameter, tubular microfibrils. Bar, 2 μm in *A* and *C*, 0.25 μm in *B* and *D*.

an important component of haemodynamics in vertebrates and provides a mechanism to convert the pulsatile flow from the heart to a reasonably smoother flow to the rest of the circulation. Elastic behaviour has now been shown to exist in the arterial wall of some lower vertebrates[3], and some invertebrates[4–7]. We demonstrate that functionally important mechanical properties are exhibited by the wall of the lamprey ventral aorta; an unusual biomaterial whose composition is based principally on the presence of microfibrils.

Materials and methods

The animals used were upstream migrant (or pre-spawning) anadromous sea lamprey, *Petromyzon mari-*

nus. All animals were anaesthetized in a 0.05% solution of tricaine methansulfonate (MS222). The head and branchial region were removed from the body at the level of the heart. The entire ventral half of the gill region was removed from the level of the first gill slit anteriorly, and to the heart posteriorly. Only the dorsal surfaces of the ventral aorta and heart were exposed, thus the ventral aorta was essentially kept in situ. The aortic segment was cannulated, and inflation-deflation cycles were generated using a syringe. The pressure changes were recorded with a modified solid state low pressure sensor (Nova Sensor, Fremont, CA), while simultaneously recording changes in the outside diameter with a Video Dimension Analyzer (Instrumentation for Physiology and Medicine, Inc., San Diego, CA) attached to a dissecting microscope. The pressure-diameter data were converted to stress-strain data with software written in Pascal using algorithms described elsewhere[3]. The elastic modulus was calculated as the slope of the inflation curve. The aortic segment was maintained in isotonic saline at 15 °C during all data collection. A total of 46 sets of inflation-deflation tests were made on aortae from six different animals, with an approximately equal number of tests per animal.

Results and discussion

Figure 2 shows a typical inflation-deflation curve for a partially isolated aorta. The mechanical hysteresis was almost 50% in this sample, indicating that almost one-half of the strain energy stored in the wall during extension was lost as heat during the recoil. This is a large hysteresis for an arterial wall; for comparison, in a study of comparative mechanical properties of aortae, the largest hysteresis was found in the wall of a snake aorta, and was only 25%. The magnitude of the hysteresis in the lamprey aorta wall was variable from one test to another, but was never larger than that indicated in figure 2. This variability was probably related to the

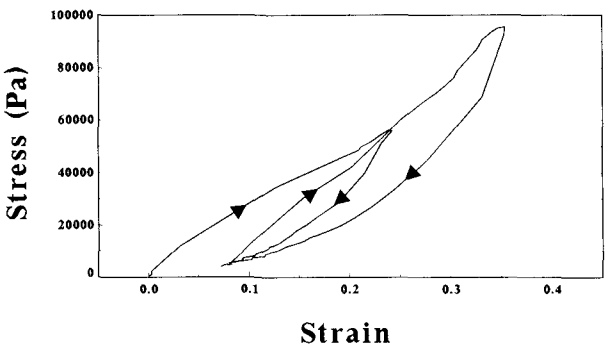

Figure 2. Plots of circumferential wall stress against strain of the ventral aorta of the lamprey. The figure shows two sequential inflation-deflation cycles. The strain rate for each loading cycle was 0.0846 and 0.0362 sec^{-1}, and the mechanical hysteresis for each complete cycle was 48% and 43%, respectively. The maximum pressure was 4931 Pa.

variability in the strain rates imposed during the tests; however, we have not attempted to quantify this relationship. Such viscoelastic phenomena are clearly important in determining haemodynamic properties, and further tests on the dynamic properties of the wall would provide useful information on energy losses at functional frequencies. However, the wall is clearly able to function mechanically as an elastic reservoir.

The non-linear elastic behaviour described above for arterial walls of higher vertebrates is functionally important in preventing local instabilities from developing at high strains[8]. We find little direct evidence of non-linear elastic behaviour in the lamprey aorta wall. Significant linear regressions of the inflation phase of the stress-strain curves were found in all tests. Visual inspection of the fitted curve and original data provided no evidence of deviations from linearity. It should be noted, however, that we have no direct measurements of in vivo ventral aorta pressures, so that a non-linear region may exist at higher pressures. Indeed, it is difficult to measure the in vivo pressure in the ventral aorta because it is surrounded laterally by gill tissue, ventrally be cartilage and the musculature and skin of the body wall, dorsally by the pharynx, notochord, spinal cord and muscle and skin of the body wall. The aorta is thus quite inaccessible without major surgery. The range of mean blood pressures shown here (table) is taken from Hardisty[9], who estimated the blood pressure in the ventral aorta from pressure measurements recorded in the dorsal aorta[10], and assuming that the pressure drop in the gill capillaries is of the same order as in teleosts (40–50%). The highest pressure we generated during a mechanical test in an isolated artery was 5706 Pa.

The lack of non-linear elastic behaviour is not surprising, since there is little collagen present in the wall to recruit as stiff elements during high strain deformations, as occurs in higher vertebrates. However, some mechanism must exist to prevent the development of aneurisms at high strains. We suggest two mechanisms that would be provided by structures located external to the artery. Firstly, the ventral aorta is completely surrounded, and intimately bound to tissues of various degrees of stiffness. Such tissues may function to limit the extensibility of the aorta at high pressures. We were not able to measure the mechanical properties of the aorta with these structures intact, since it would restrict our ability to measure diameter changes during the inflation-deflation tests. Secondly, the lamprey heart is surrounded by an unusually rigid closed pericardium that might restrict the magnitude of cardiac muscle contractions.

Comparisons of mechanical and physiological data between various vertebrate aortae are instructive; the table summarizes some comparative data. The elastic modulus for a lamprey aorta wall at physiological pressures is lower than the more advanced vertebrates listed. This result is not surprising, since the compositions of the

Comparative properties of vertebrate aortae. Data for all the species except the lamprey are from other sources[3]. The elastic moduli for the first four species were measured at the mean blood pressure.

Species	External radius (mm)	Relative wall thickness	Mean blood pressure (kPa)	Elastic modulus (MPa)
Toad (*Bufo marinus*)	0.9	0.11	3.0	0.33
Lizard (*Gekko gecko*)	0.6	0.14	3.0	0.30
Snake (*Thamnophis radix*)	0.7	0.12	4.4	0.51
Rat (*Sprague-Dawley* strain)	1.1	0.2	11.0	0.5
Lamprey (*Petromyzon marinus*)	1.5	0.18	4.0–8.0	0.23

arteries are different. Maximum strains (not included in the table) were typical of the value shown in figure 2, indicating that the wall is less compliant at physiological pressures than the aortic walls of more advanced vertebrates.

We conclude that the architecture of the lamprey ventral aorta provides a mechanical system that produces sufficient elastic behaviour to act as a reservoir to damp out heart pressure fluctuations, and that the development of instabilities in the wall at high strains are probably restricted by structures external to the artery. This is the first time that a biomaterial whose composition is based principally on the presence of microfibrils has been shown to have functionally important mechanical properties. It is interesting to note that morphological studies of elastogenesis show that developing elastin fibres are initially composed solely of microfibrils, and the mature elastin fibre consists of amorphous elastin as the main component with some peripheral microfibrils.

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