V-shaped cushion at the origin of bovine pulmonary supernumerary arteries: structure and putative function

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V-shaped cushion at the origin of bovine pulmonary supernumerary arteries: structure and putative function. J. Appl. Physiol. 87(6): 2348–2356, 1999.—This study investigates the anatomic structure at the origin of pulmonary supernumerary arteries and their parent conventional artery. Histological examination showed that at the origin of each supernumerary artery the wall of the parent conventional artery is organized into a distinct V-shaped structure, which begins on the hilum side of each supernumerary artery as a funnel-shaped channel running into the supernumerary artery. The base of the channel is particularly thin walled. The lateral walls of the channel are composed of musculoelastic cushions that become more pronounced toward the supernumerary artery and fuse on its distal side, forming a baffle that projects over the supernumerary artery lumen. These V-shaped structures/cushions were observed with video stereotop dissecting microscopy in both an open and closed state in isolated arteries in vitro. Pulmonary vasoconstriction of isolated arteries with the thromboxane A2 mimetic U-46619 increased the number of V-shaped structures in the closed state. These studies indicate the presence of a novel anatomic structure at the origin of pulmonary supernumerary arteries, which may be able to regulate blood flow into the supernumerary artery.

pulmonary vascular bed; pulmonary vascular resistance; recruitment; baffle valve; pulmonary hypertension

MATERIALS AND METHODS

Bovine lungs from 20 cattle over 18 mo of age were obtained from a local abattoir within 20 min of slaughter and transported to the laboratory in ice-cold Krebs physiological saline solution (PSS) of the following composition (in mM): 140 NaCl, 4.7 KCl, 24.8 NaCO3, 1.2 MgSO4, 1.2 KH2PO4, 2.5 CaCl2, and 11.1 glucose.

Dissection. In both the right and left lungs, the third-generation pulmonary artery formed the axial artery. From this artery, the parenchyma and peribronchiolar connective tissue on the surface not opposing the bronchi were carefully removed so that the branching pattern could be visualized. This revealed an axial artery 150–200 mm in length, having a diameter of 20–25 mm at its proximal end and 3–5 mm at its distal end. The majority of branches arising from the axial artery were at 90° (monopody), with fewer branches arising at an oblique angle (dichotomy). The supernumerary arteries were identified as those vessels arising at −90° and with no associated airway.

Segments of axial artery (20 mm in length), containing one or more side branches of 2–3 mm in length, were dissected out by using microdissecting scissors. Care was taken not to stretch or damage the tissue. The isolated tissue samples were kept in fresh ice-cold PSS buffer before use.

Histology. Conventional and supernumerary arteries were fixed in neutral-buffered Formalin. After dehydration in graded alcohol, the tissues were embedded in paraffin wax. Sections (20 µm) from these specimens were stained by using Miller’s (11) and van Gieson’s (15) stains to demonstrate the distribution of elastin and collagen, respectively. The stained sections were examined and photomicrographs taken by using a Zeiss Axiophot microscope.

Pharmacology. For studies showing the luminal surface of the conventional artery, segments of conventional artery were dissected out as described above and cut along the longitudinal axis. The artery was then pinned out on an 8-in. petri dish with a charcoal-impregnated silicon (silgard) base. The tissue was covered with PSS, maintained at 37°C, and gassed with 95% O2-5% CO2. The tissue was allowed to equilibrate
for 1 h under these conditions before the addition of the thromboxane A<sub>2</sub> mimetic U-46619 (100 nM). A total of five experiments were conducted to examine the effect of U-46619. The luminal surface was illuminated by a cold-light source (Schott, KL 1500) and visualized by a stereo dissecting microscope (Leica, Wild M32). Images were recorded by using a JVC video camera (TK-885E) and video recorder (Panasonic, NV-HS800B).

Confocal microscopy. Segments of conventional or supernumerary arteries were dissected as described above. Artery segments were incubated in the dark at room temperature with the vital nuclear stain Hoechst 33342 (1 mg/ml) for 60 min (3). After four washes in fresh PSS, segments were cut open and placed, lumen side up, in the well of a glass slide. The greased well was flooded with fresh PSS, and a coverslip was placed on top. Slide-mounted artery segments were then visualized by using laser-scanning confocal microscopy (Odyssey XL; Noran Instruments). The Odyssey scan module was fitted to a Nikon Optiphot microscope (Objective; ×40 oil, NA 1.3). Excitation of Hoechst 33342 was achieved with the 364-nm line of an ultraviolet argon-ion laser (long pass, 400 nm). For image collection, identical levels of laser intensity (100%), acousto-optic deflector power, brightness (photomultiplier tube offset), and contrast (photomultiplier tube gain) were used. Digital images were collected by using a 64-frame average to reduce noise. Smooth muscle cells were identified as being those cells the nuclei of which lay beneath the (autofluorescent) internal elastic lamina. The orientation of the smooth muscle cells was determined from the long axis of the endothelial cell nuclei. Images of smooth muscle cell nuclei were collected at intervals of 0.5 µm along the axial plane (z-axis) to produce an image stack. A series of images (the stack) was then combined to produce an extended focus view of all nuclei in all planes.

Fig. 1. Dissection of bovine lung exposing 3rd-generation conventional artery (axial artery). Length of exposed artery is 150 mm. Supernumerary artery side branches are shown by arrowheads. Bar = 2 cm.

Fig. 2. Transverse section of a supernumerary artery (SA) and associated small muscular artery (arrow) (stain: Miller’s elastin and van Gieson). Bar = 100 µm.
RESULTS

Gross morphology. Figure 1 illustrates a dissection of the bovine lung with the third-generation conventional pulmonary artery (axial artery) exposed. The supernumerary artery side branches, which arise from the conventional artery at 90°, can be seen clearly. Approximately 8–10 small vessels originate at 90° from a 30-mm-long section of this artery. The branches arising at right angles from the axial artery measured 800–1,000 µm in diameter at the proximal end and 50–200 µm in diameter at the distal end. On a few occasions, the side branches supplied a small bronchiole arising from the axial bronchus or, more commonly, divided rapidly within 2–10 mm of their origin to supply the parenchyma adjacent to the axial artery. The branches arising obliquely from the parent artery followed the underlying airway and continued to produce oblique and right-angled branches of decreasing diameter toward the periphery. The dichotomous branches varied greatly in size ranging from 20 to 100% of the axial artery diameter. The ratio of right-angled branches to oblique branches increased from 6:1 at the proximal end to 8–10:1 at the distal end. Each supernumerary artery was associated with one or more small arteries (Fig. 2).

At the origin of each supernumerary artery, on the proximal side, the wall of the conventional artery is composed of a V-shaped cushion, with the open end facing into the direction of blood flow. The luminal surface of the conventional artery is highly folded along the longitudinal axis. These folds extend into the distal part of the V-shaped cushion. The wall within the V-shaped cushion (channel wall) is also highly folded (Fig. 3).

Histology. The wall of a conventional artery in cross section immediately preceding the start of the V-shaped cushion is illustrated in Fig. 4A. The V-shaped cushions arise at the proximal side of the supernumerary artery as a thinning of the wall of the conventional artery (Fig. 4B). The thin-walled region takes on the form of a distinctive channel, and toward the distal part of the V-shaped cushion the channel becomes narrower, deeper (Fig. 4, B and C), and, eventually, continuous with the wall of the supernumerary artery (Fig. 4E).

The medial layer forming the lateral walls of the channel has the appearance of distinct cushions. The cushions become progressively more pronounced, with a denser elastin content toward the distal part of the V-shaped cushion, and protrude into the channel (Fig. 4, B-D). Eventually, the cushions on either side of the channel become continuous, forming the distal part of the V-shaped cushion (Fig. 4, E and F).

The distal part of the V-shaped cushion has a dense content of elastin, and it appears to take the form of a lip or baffle (Oxford English Dictionary definition of baffle: a plate that can be adjusted to direct flow) extending in a proximal direction over the supernumerary artery (Fig. 5A). The internal elastic lamina is highly folded in this region (Fig. 5B).

Figure 6, A and B, shows transverse sections of the conventional artery illustrated in Fig. 4 and the point at which the two cushions on either side of the channel fuse to form the baffle region of the V-shaped cushion. These sections illustrate the musculoelastic nature of this region of the V-shaped cushion. They also illustrate the extent to which the longitudinal folds in the conventional artery, shown in Fig. 3, extend into the baffle region of the V-shaped cushion.

A confocal image (extended focus) of smooth muscle-like nuclei in the channel cushion shows two distinct orientations of smooth muscle cells in this region. The muscle layer closest to the lumen is oriented in the longitudinal axis of the conventional artery. The deeper layer has the same orientation as the normal circular muscle (Fig. 7).

Figure 8 is a simplified schematic drawing illustrating the principal features of the V-shaped cushion.

Pharmacology. In any particular conventional artery, in the absence of vasoconstrictors, examples of both
“open” (Fig. 9A) and “closed” (Fig. 9C) V-shaped cushions can be observed.

A single video frame of a conventional artery is shown before and after the exposure to the vasoconstrictor U-46619 (100 nM). Figure 9A shows the artery before exposure to the thromboxane A2 mimetic U-46619 (100 nM). The addition of U-46619 narrowed the conventional artery, closed the smaller V-shaped cushions completely, and partly closed the larger V-shaped cushions (Fig. 9B).

**DISCUSSION**

This study in the bovine lung supports previous findings in the human (4) and rat (7) that the “great majority” of divisions from the conventional artery are at ~90° (monopodic division) and is also in agreement with our own observations in the pig and sheep (unpublished observations). Whereas some of these vessels are accompanied by a small airway, the majority are not accompanied by an airway and divide rapidly to supply gas-exchange units in their immediate vicinity.

A number of new findings are shown by the present study.

Supernumerary arteries in the bovine lung. In the bovine lung, we found a slightly greater supernumerary artery-to-conventional artery ratio than that reported by previous studies in the human; i.e., 6–8 supernumerary arteries per conventional artery near the hilum, increasing to 8–10 supernumerary arteries...
per conventional artery toward the periphery. There appear to be species differences in the supernumerary artery-to-conventional artery ratio. In the rat lung, there are equal numbers of supernumerary and conventional arteries (7). The relatively greater number of supernumerary arteries in the bovine lung may be related to the more distinct lobulation in this species (1).

The present study also shows that small arteries are associated with supernumerary arteries. Because these small vessels were seen to give off branches to the supernumerary artery wall (unpublished observations), it is likely that they are vasa vasorum derived from the bronchial circulation that provides a nutrient blood supply to the supernumerary artery.

V-shaped cushions. This study reports the presence of a musculoelastic structure at the origin of the supernumerary artery in the bovine lung and shows that the structure is not a circular sphincter but has a distinct V shape.

In the bovine lung, on the proximal side of each supernumerary artery, the wall of the conventional artery is organized into a funnel-like channel running into the supernumerary artery. Each side of the channel is bound by a pronounced cushion that projects into,

![Image 1](image1.png)

**Fig. 5.** Longitudinal section of CA at origin of a SA (stain: Miller’s elastin and van Gieson). A: B structure formed by cushions on the distal wall of SA. Arrow shows direction of blood flow in the CA (bar = 110 µm). B: baffle-like structure at a high magnification, illustrating folded nature of internal elastic lamina in the cushion and at origin of SA. Bars = 50 µm.

![Image 2](image2.png)

**Fig. 6.** Transverse section of a CA at origin of a SA at a point where the 2 cushions of V-shaped cushion become continuous to form B structure. A: shows dense elastin content of this region and the extent to which longitudinal folding (arrow) in wall of CA extends into baffle region (stain: Miller’s elastin and van Gieson). Bar = 190 µm. B: shows muscular nature of V-shaped cushion and the extent to which baffle region is folded (arrow) (stain: hematoxylin and eosin). Bar = 45 µm.
and forms the upper part of the channel. The cushions lining either side of the channel fuse on the distal side of the supernumerary artery (producing the V shape) forming a bafflelike structure on the distal wall of the supernumerary artery. The V-shaped cushions have a dense, although diffuse, elastin content, and lying between the endothelium and the circular smooth muscle is a band of longitudinal muscle.

The musculoelastic structure, reported to be a circular sphincter in the human lung (4), was only visualized in cross section and has a similar appearance to the V-shaped cushion when viewed in cross section. It is possible, therefore, that the structure in the human lung may have been mistakenly interpreted as a circular sphincter and that it may, in fact, be similar to the V-shaped cushion in the bovine lung.

The V-shaped cushions appear to belong to a group of related structures found at the origin of arterial branches (6, 8–10, 12, 16). In their commonest form, these structures appear to consist of a pair of streamlined cushions, composed of longitudinal muscle, that are located on either side of the branch origin. In these paired structures, the two cushions are fused at either end to give a lip-like appearance. The V-shaped cushions have a similar structure to the paired cushions but appear to be the least common in the tissues that have been investigated previously.

The V-shaped cushions described in this study are essentially similar to those described by Mark (9, 10) in avian arteries and by Moffat (12) in the uterine artery from the rat. In particular, the serial transverse sections illustrated in Fig. 4, showing the formation of the cushions and the channel, are very similar to those illustrated by Mark in avian mesenteric artery (9, 10).

Putative function of the V-shaped cushion. It is generally believed that the function of paired cushions is in the regulation of blood flow into the branch (6, 8–10, 12, 16). In this context, it has been suggested that a contraction of the longitudinal muscle would increase the bulk of the cushion, reducing the lumen diameter at the origin, and, as a consequence, restrict flow into the branch (12). Functional evidence for the role of arterial cushions in the regulation of blood flow is provided by the observation that contraction and relaxation of arterial cushions correlate with periods of minimal and maximal, respectively, blood flow in the rat uterus (8).

The main characteristics of the V-shaped cushions, the funnel-like channel together with the baffle-like structure on its distal part, give the overriding impression that the V-shaped cushions act to direct blood into the supernumerary artery. This role may be related to the fact that supernumerary arteries arise from conventional arteries at a right angle, a feature that would surely discourage smooth blood flow into the supernu-

Fig. 7. Confocal image of fluorescent nuclei from the cushion of V-shaped cushion (Hoechst 33342 was used to stain nuclei). Image shows smooth musclelike nuclei in 2 distinct orientations. Longitudinal (L; arrow) smooth muscle nuclei closest to lumen are oriented in the longitudinal axis of vessel and have same orientation as larger endothelial cell nuclei (not shown). A deeper layer of circular (C; arrow) smooth muscle is indicated, with nuclei oriented at 90° to the endothelial cell nuclei.
merary arteries. This possibility is supported by the fact that in the uterine artery the V-shaped cushions, unlike the paired cushions, were only found at arterial branches that arose at a right angle (12). Therefore, it is possible that the V-shaped cushion may be an adaptation that channels blood into arterial branches, such as the supernumerary arteries, that arise at a right angle.

It is clear that some sections of the same conventional artery contain V-shaped cushions that appear to be in an “open state,” or at least partly open, so that the entrance to the supernumerary artery is visible to some extent. In other sections, the V-shaped cushions appear to be in a “completely closed state,” so that the supernumerary artery cannot be seen. In all of the conventional arteries that we examined, we found single V-shaped cushions or groups of V-shaped cushions that were in the “fully closed position.” Stretching the conventional artery wall across its diameter pulled these closed V-shaped cushions to an open state, exposing the channel and the supernumerary artery lumen. We did not extend this study to estimate the ratio of open to closed V-shaped cushions in the bovine lung. However, both Mark (9, 10) and Moffat (12) also reported the presence of open and closed V-shaped cushions and reported that the closed state was the most common state found in their vessels.

The present study also shows that active vasoconstriction of the conventional artery increases the number of V-shaped cushions in the closed state. These observations indicate that V-shaped cushions may be regulated and that regulation may be related to the diameter of the conventional artery. This may suggest that, because tone is normally greater in vivo than in vitro, a greater number of V-shaped cushions would be in a closed state in vivo. Also, since luminal pressure appears to be linearly related to diameter (2), changes in the diameter of the conventional artery that arise from changes in blood pressure may also be important in regulating V-shaped cushions.

It is clear from this study that the shape of the V-shaped cushions can be altered to produce a more open or closed state. The observation that the closed state appears to occlude the supernumerary artery lumen raises the possibility that the V-shaped cushion may act as a “baffle valve” to regulate flow into the
Fig. 9. A: single video frame showing luminal surface of a 4th-generation CA. Frame shows origin of 6 SAs and their associated V-shaped cushions. Arrow indicates direction of blood flow. Bar = 600 µm. B: single video frame of artery segment shown in A 90 s after addition of vasoconstrictor U-46619 (100 nM). Bar = 600 µm. C: single video frame from a different section of the 4th-generation CA shown in A. Frame shows origin of 3 SAs where V-shaped cushions are in a closed state (arrowheads). Bar = 370 µm.
supernumerary artery. This possibility remains to be demonstrated. However, there are several observations that are of interest in this context. First, in the dog lung, Fillenz (5) reported that the origin of supernumerary arteries was particularly densely innervated, which suggests that this region is important in regulating flow. Second, in the human lung in vivo, Elliott and Reid (4) reported that most supernumerary arteries, and some monopodic conventional arteries, were not visible on pulmonary angiograms. Although it may be argued that this indicates a lower uptake of the dye by these vessels or simply a technical artifact related to the resolution permitted by the angiography technique, the more peripheral supernumerary arteries, which lack a musculoelastic structure, were visible in these angiograms.

V-shaped cushions in pulmonary hypertension. In pulmonary hypertension, some of the most marked pathological changes are reported to occur at the origin of the supernumerary artery. For example, medial proliferation appears to be particularly pronounced at the cushions, resulting in the formation of a "slitlike lumen" or complete obstruction of the branch lumen (17). This effect is also elegantly illustrated by Yaginuma et al. (18) and may indicate that smooth muscle proliferation in the V-shaped cushion is particularly important in reducing the cross-sectional area of the pulmonary artery bed in pulmonary hypertension.

Dilation lesions (17) are reported to be located almost exclusively at the origin of supernumerary arteries (13, 18). Because the thin-walled channel is likely to experience the greatest stress under elevated arterial pressure, it seems likely that the channel may be the initial site of the focal dilation.

In conclusion, the present study shows that the anatomic structure at the origin of bovine pulmonary supernumerary arteries is a V-shaped musculoelastic cushion located on the proximal side of the supernumerary arteries. The present study also indicates that a pulmonary vasoconstrictor closes the V-shaped cushions, suggesting that this structure is able to regulate blood flow into supernumerary arteries. Because the distal part of the V-shaped cushion may act like a baffle, we have referred to the V-shaped cushion as a baffle valve.

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