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Pulmonary Vascular Smooth Muscle and Its Interaction with Endothelium*

Morphologic Considerations

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In pulmonary arteries the medial smooth muscle cells and endothelial cells, though separated by an internal elastic lamina, come into contact by way of fenestrations in this lamina. Such a continuous elastic lamina is absent in pulmonary veins facilitating this contact. If vasoconstriction is induced in experimental animals, herniations of medial smooth muscle cells protruding through endothelial cells provide an extensive and close association and thus a potential interaction between both cell types. This is particularly prominent in the veins. In arteries these herniations, which must penetrate the fenestrations in the elastic lamina, are far less conspicuous. Intimal fibrosis, for instance, as an age change, is not necessarily an impediment for such interaction, since the cells within the intimal layer have all the characteristics of smooth muscle cells and thus may provide a smooth muscle-endothelium contact.

The contribution of the morphologist to the problem of interaction between pulmonary vascular smooth muscle cells and endothelium is of necessity limited. The morphology of these cells, particularly their topographic relations, may suggest the likelihood or the improbability that such interactions exist, but an actual mutual influence can not easily be demonstrated or excluded in this way. Even so, the study of the morphologic aspects may contribute to the understanding of an eventual interaction.

In normal lung vessels the intima is extremely thin, consisting mainly of a single layer of endothelial cells overlying a basement membrane. The endothelial cytoplasm forms a layer of approximately 0.2 to 0.5μ thickness with intercellular junctions between adjacent cells. At the sites of the endothelial nuclei the cells are somewhat thicker, producing mild protrusions into the lumen. There are prominent differences between the muscular pulmonary arteries and veins of comparable size that may well have implications with regard to smooth muscle-endothelium interaction.

In normal muscular pulmonary arteries the media is thin, consisting of a layer of smooth muscle cells with an approximately circular arrangement with narrow intercellular spaces, containing scarce reticulin fibers. Thus, although endothelial cells and medial smooth muscle cells are almost adjacent, they are still separated by an elastic lamina of variable thickness. This internal elastic lamina, however, contains multiple small fenestrations, approximately 0.5 to 1.0μ wide. Short protrusions of both endothelial and muscular cytoplasm can be seen extending through these fenestrations (Fig 1).

In the normal pulmonary veins the media is even thinner than in the arteries. It consists of irregularly arranged smooth muscle cells separated by much more collagen and ground substance than in the arterial media. However, there are usually many elastic fibers within the media. Also, between the intima and media there are elastic fibers, but even when these form some sort of lamina, it is discontinuous, allowing ample contact between endothelium and smooth muscle.

Striking changes in this mutual arrangement occur in vasoconstriction. We induced vasoconstriction of pulmonary arteries in three ways: in rats by hypoxia and by administration of fulvine, one of the Crotalaria alkaloids. By either method pulmonary hypertension is produced, shown by

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right ventricular hypertrophy and by medial hypertrophy of the pulmonary arteries. This becomes recognizable after one week. With fulvine, however, contraction apparently is more intense and spastic than in hypoxia, since it leads to fibrinoid necrosis of pulmonary arteries and eventually death of virtually all animals thus treated. Even so, the early changes observed following either method are largely the same when vasoconstriction ensues. In addition, we perfused isolated lungs of guinea pigs with histamine, which led to an almost instantaneous vеноconstriction with an associated increase in pulmonary arterial pressure.3

In all these models constricted pulmonary vessels have a narrow lumen and a relatively thick media. In arteries the internal elastic lamina is crenated. In collapsed lung tissue this lamina also regularly shows some crenation, but when the lungs are expanded by intratracheal instillation of Karnovsky's fixative, the outline of the internal elastic lamina is smooth in control animals but remains crenated in animals subjected to vasoconstriction.

In electron micrographs contracted smooth muscle fibers show excrescences or herniations of the cytoplasm, as long as their formation is not inhibited by adjacent structures, so that the surface of the cells may no longer be smooth. Inasmuch as the muscle cells border on the internal elastic lamina, these excrescences tend to lie in the folds of this lamina. Regularly, however, some of them protrude through fenestrations of the elastic lamina so that they are in immediate contact with the endothelium while, with a thin stalk, they are continuous with the main body of the muscle cell. Within the herniations that arise from the sarcolemma between two points of insertion of myofibrils, the cytoplasm is pale, virtually devoid of myofibrils and with scanty organelles, as opposed to the cytoplasm of the main cell body.1,4

If there should be any doubt whether these muscular excrescences really represent smooth muscle cell contraction, we may refer to the work of Fay and Delise,4 who demonstrated beautifully that numerous cytoplasmic excrescences occur in isolated smooth muscle cells from the stomach of the toad in response to electrical stimulation. These excrescences show the same pale cytoplasm with scarce organelles shown in our studies.

Remarkably, smooth muscle excrescences are far more striking in pulmonary veins. This undoubtedly results from the absence of a more or less continuous internal elastic membrane in these vessels. This permits a more free formation of the herniations and an easier contact with the endothelium, although no intercellular junctions or other surface specializations are formed between these cells (Fig 2). The herniations in the veins are numerous and often large. They may cause indentations in overlying endothelial nuclei. In severe contraction, as caused by fulvines, they may push the thin layer of endothelial cytoplasm up into the lumen, sometimes forming blebs hanging in the lumen. It seems likely that such blebs may become detached and carried away by the bloodstream.

It must be stressed that the muscular cytoplasm herniations enhance the contact between smooth muscle and endothelium in two ways. The shortest distance between the two cell types is decreased, and the area of contact is considerably increased. This may indicate that contraction of a vessel could well promote a more active interaction between smooth muscle and endothelium.

What happens in sustained pulmonary hypertension with regard to this contact, particularly when intimal changes develop? In many forms of pulmonary hypertension intimal
fibrosis, although varying in appearance, may be very pronounced. Such a layer of intimal fibrosis means that there is no or very little contact between medial smooth muscle cells and endothelium. Even if muscular herniations should penetrate the internal elastic lamina under these circumstances, they generally will not reach the endothelial cells. Does that mean that an eventual interaction is excluded in these instances? It certainly is possible. On the other hand, it is important to realize that intimal fibrosis also occurs in the absence of an elevated pressure. In perfectly normal adult individuals over the age of 40 or 50 years, there is often a thin layer of intimal fibrosis as an age change not only in pulmonary arteries but also in pulmonary veins. Whatever the benefit of an eventual smooth muscle-endothelium interaction, it would be a sobering thought if it does not apply to middle-aged and older people.

However, there is perhaps some hope for these age groups and for others afflicted by intimal thickening. The layer of intimal fibrosis, as the name implies, contains ground substance with collagen and, to a lesser extent, elastic fibers. But the cells within this layer have almost all the ultrastructural characteristics of smooth muscle cells, even though it is impossible to recognize them as such under the light microscope. Their cytoplasm contains densely packed myofilaments with fusiform electron-dense bodies. There are many attachment sites along the plasma membranes, and often there are micropinocytic vesicles. The only difference with the smooth muscle cells in the media is that the latter are essentially elongated and lie close together, whereas the intimal cells are more irregular and, even when oval or elongated, have several cytoplasmic protrusions in various directions, while they are widely separated by ground substance and collagen. Inasmuch as they are adjacent to the endothelial layer, they make contact with the endothelial cytoplasm by way of small protrusions (Fig 3). There is evidence that the intimal cells are derived from the media. In fact, cells are regularly lying half in the media and half in the intima, squeezed in the middle within a narrow fenestration.

There are many forms of intimal thickening, dependent on the type of pulmonary hypertension. There is even a form in which the intimal thickening consists of densely packed longitudinal smooth muscle cells that can be recognized under the light microscope. In all forms of intimal fibrosis, the cells within the intima have the ultrastructural features of smooth muscle cells. Since the intimal cells border freely on the endothelium, not even hindered by an elastic lamina, some sort of smooth muscle-endothelium interaction may even be possible in the presence of intimal thickening.

We fully realize that we have not produced any indication that such an interaction exists. This is a task for other disciplines. We hope, however, to have demonstrated that the morphology and topographic contact between smooth muscle and endothelium do not stand in the way of a physiologic interaction, particularly in the presence of vasoconstriction.

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DISCUSSION

An unresolved controversy exists as to the optimal method to fix a lung for histologic examination. There was agreement among the participants that if the vessels are to be examined, they should not be injected. Whether lungs are prepared by rapid freezing or transbronchial fixation, normal vessels appear to be completely expanded. However, there was disagreement as to the extent to which fixation via the airways might alter the structure existing in life in hypertensive vessels.

The question was raised whether herniations of smooth muscle through the internal elastic lamina occurred during acute hypoxic pulmonary vasoconstriction. The herniations are apparent following the acute administration of histamine and after chronic vasoconstriction caused by fulvulne. The herniations always occur between two attachment sites of the myofilaments and may represent cytoplasm streaming out between the two attachments. It was considered that the electron micrographs shown supported the conclusion that the contractile filaments run longitudinally in the smooth muscle cell. The herniations are not thought to be an artifact of fixation or to be composed of edema fluid. Herniations also occur in smooth muscle cells distant from the elastic laminae.

It was suggested that the herniations might provide a pathway for the endothelial relaxing factor to pass from the endothelium to the smooth muscle. However, the point was made that even when an artery is relaxed and presumably herniations are not present, the administration of acetylcholine causes a large increase in cyclic GMP.

There are numerous fenestrations in the internal elastic lamina allowing contact between the endothelial and smooth muscle cells. There does not seem to be a specialized structure at the point of contact, and it was suggested that they may merely transmit mechanical forces. A discussant reported that in one study over 125 points of contact were counted in pulmonary arterioles 200 μ in diameter on electron micrographic thin sections. An additional feature of the smooth muscle cells is that they can contain even more caveolae than the endothelium. The function of these caveolae is unknown. In the thickened intima observed in the presence of pulmonary hypertension, the smooth muscle cells are considered to have migrated through the internal elastic lamina.