Communicating junctions between pillar cells in the gills of the atlantic hagfish, *Myxine glutinosa*[1]

H. Bartels and B. Decker

Laboratory of Electron Microscopy, 4130, Hannover Medical School, D-3000 Hannover (Federal Republic of Germany),
6 August 1984

Summary. In the respiratory lamellae of the gills of the atlantic hagfish, *Myxine glutinosa,* pillar cells are coupled by communicating junctions. It is suggested that these cells behave like a functional syncytium.
Key words. Gills; hagfish, *Myxine glutinosa;* pillar cells; communicating junctions; freeze-fracture.

The structural organization of the secondary or respiratory[2] lamellae is essentially the same in various groups of fish[3], including recent Agnathans[4,5], which are represented by hagfish and lampreys, although the general organization of the gills is markedly different in these jawless vertebrates and gnathostome fish[6]. In the respiratory lamellae a central blood space is separated from the water by squamous, flange-like processes of the pillar cells, the two-layered flat gill epithelium and an intervening basement membrane. Regularly-spaced posts, extending between the opposite epithelial cell layers, transversely cross the blood space. These posts are composed of the cell bodies of the pillar cells which completely surround bundles of collagen fibrils and elastic microfibrils ('columns'). In this study on the gills of the atlantic hagfish, *Myxine glutinosa,* the pillar cells were investigated by freeze-fracture.

Materials and methods. Myxine glutinosa were obtained from the Drøbak Biological Station, Norway, and kept under conditions described in detail elsewhere[7]. The gill lamellae were cut free from the outer wall of the gill pouch, fixed in phosphate-buffered (0.1 M, pH 7.1) or Na-cacodylate-buffered (0.1 M, pH 7.4) glutaraldehyde (6%) overnight at 4°C, cryo-protected in glycerol (30%) in saline for 1–2 h at room temperature and processed for freeze-fracture according to the standard preparation protocol[7]. EM: Siemens Elmiskop IA, 80 kV.

Results. In freeze-fracture replicas of the respiratory lamellae, the pillar cells were identified by their location internal to, but close to the prominent epithelial basement membrane and by their intimate association with bundles of collagen fibrils (fig. 1). Small clusters of tightly packed particles (8–9 nm diameter) on the P-face (fig. 2) and of pits on the E-face of the split plasma

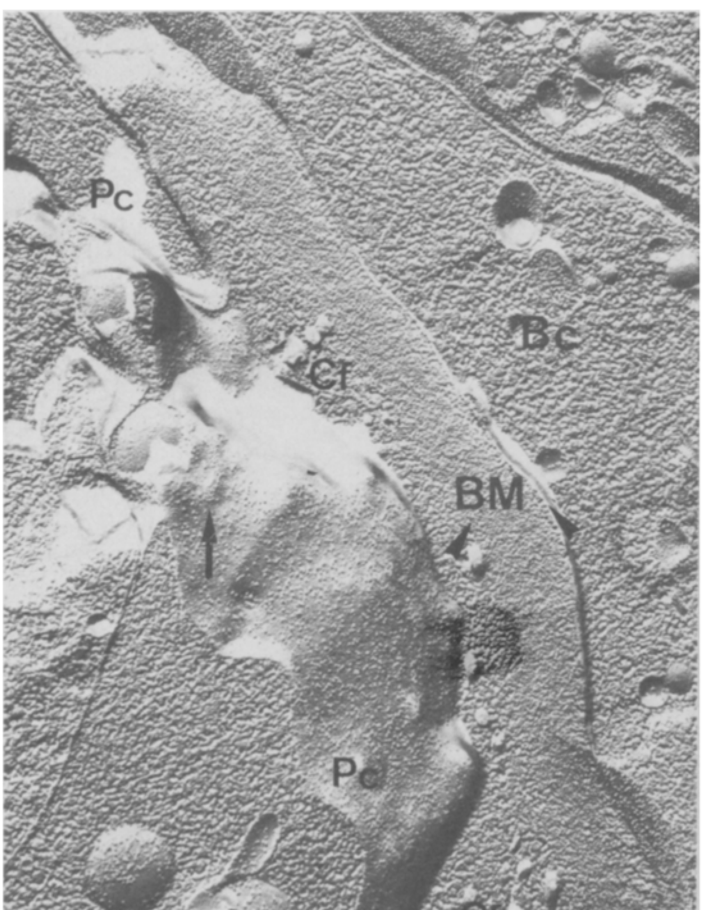

Figure 1. Respiratory lamellae of the hagfish gill. On the right a basal cell (Bc) of the gill epithelium, on the left two pillar cells (Pc). Underneath the epithelium the felt-like basement membrane (BM); between the basement membrane and the pillar cells collagen fibrils (Cf). The arrow points to a communicating junction, enlarged in figure 2. ×40,000.

Figure 2. Communicating junction on the P-face of the split plasma membrane of a pillar cell. ×160,000.

membrane of the pillar cells were occasionally observed. They were considered to represent communicating junctions[8].

Discussion. Evidence is presented from freeze-fracture replicas that the pillar cells in the respiratory lamellae of the gills of the hagfish are connected by communicating junctions.

The pillar cells share structural and functional characteristics with smooth muscle-like cells (myofibroblasts), such as the renal mesangial cells[9] and the interstitial cells in the interalveolar septa of mammalian lungs[10]. In contrast to the pillar cells these cells are separated from the circulating blood by an original endothelium. However, each of the three cell types is considered to be involved in the regulation of the local blood flow through the corresponding microcirculation at the capillary, i.e. postarteriolar, level, as they are supplied with contractile filaments[10-12]. The three cell types appear also to be responsible for the synthesis and secretion of collagen fibrils and noncollagenous compounds of the extracellular matrix[3,9,10,13-15]. In addition, by freeze-fracture the mesangial cells[16,17] and the interstitial cells in the interalveolar septa of the lungs[18] have been shown to be coupled by communicating junctions. Numerous correlative functional and morphological studies indicate that communicating junctions are the sites where electrotonic and/or metabolic coupling can take place by intramembrane, transcellular, low-resistance pathways[19,20]. The observations reported here suggest a coupling between the pillar cells in the hagfish gills. Thus the communicating junctions between the pillar cells could also allow a synchronized reaction of these cells and thereby facilitate a putative local regulation of the lamellar perfusion as previously discussed for the renal mesangial cells[17] and for the interstitial cells in mammalian lungs[18]. The contraction of the pillar cells is generally considered to be under the control of humoral factors[21], but, although it is disputed[3,21], a direct innervation of the pillar cells has been suggested by Gilloteaux[22] for the secondary lamellae of the gills of the eel.

Moreover, a similar mechanism to that which has been proposed for mammalian lungs[10,23], by which the regional blood flow is regulated by the external oxygen tension, may be operating in the respiratory lamella of fish gills.

Finally, a coordination of the metabolic and secretory activity of the pillar cells, concerning their fibroblast-like functions, could also be achieved by the communicating junctions, as has been shown for various fibroblasts and other secretory cells[20,24].

1 Supported by the DFG, SFB 146. The authors are indebted to Drs F. Walvig and H. Stolte for kindly providing the hagfish. The expert technical assistance of G. Voss-Wermbter, Dipl.-Ing., and H. Heidrich is gratefully acknowledged.

2 In this paper the term 'respiratory lamellae' is preferred to 'secondary lamellae' emphasizing the functional similarity of these structures.

3 Hughes, G.M., and Morgan, M., Biol. Rev. *48* (1973) 419.
4 Rauther, M., Gegenbaurs morph. Jb. *75* (1935) 613.
5 Youson, I.H., and Freeman, P.A., J. Morph. *149* (1976) 73.
6 Starck, D., Vergleichende Anatomie der Wirbeltiere auf evolutionsbiologischer Grundlage. Springer-Verlag, Berlin, Heidelberg, New York 1979.
7 H. Bartels, Anat. Rec., in press (1985).
8 McNutt, N.S., in: Dynamic aspects of cell surface organization, p. 75. Eds G. Poste and G.L. Nicolson. Elsevier/North-Holland Biomedical Press, Amsterdam, New York, Oxford 1977.
9 Latta, H., Biol. cell. *39* (1980) 245.
10 Kapanci, Y., Assimacopoulos, A., Irle, C., Zwahlen, A., and Gabbiani, G., J. Cell Biol. *60* (1974) 375.
11 Becker, C.G., Am. J. Path. *66* (1972) 97.
12 Bettex-Galand, M., and Hughes, G.M., J. Cell Sci. *13* (1973) 359.
13 Burke, J.M., and Ross, R., Int. Rev. conn. Tissue Res. *8* (1979) 119.
14 Pickrell, J.A., Lung Connective Tissue: Location Metabolism and Response to Injury. CRC Press, Inc., Boca Raton 1981.
15 Gabbiani, G., and Montandon, D., Int. Rev. Cytol. *48* (1977) 187.
16 Pricam, C., Humbert, F., Perrelet, A., and Orci, L., J. Cell Biol. *63* (1974) 349.
17 Kühn, K., Reale, E., and Wermbter, G., Cell Tissue Res. *160* (1975) 177.
18 Bartels, H., Am. J. Anat. *155* (1979) 125.
19 Bennett, M.V.L., and Goodenough, D.A., Neurosci. Res. Prog. Bull. *16* (1979) 373.
20 Perrachia, C., Int. Rev. Cytol. *66* (1980) 81.
21 Laurent, P., and Dunel, S., Am. J. Physiol. *238* (1980) R147.
22 Gilloteaux, J., Experientia *25* (1969) 270.
23 Kapanci, Y., MoCostabella, P., and Gabbiani, G., in: Lung Cells in Disease, p. 69. Ed. A. Bouhuys. Elsevier/North-Holland Biomedial Press, Amsterdam, New York, Oxford 1976.
24 Hooper, M.L., and Subak-Sharpe, J.H., Int. Rev. Cytol. *69* (1981) 45.

0014-4754/85/081039-02$1.50 + 0.20/0

Elastase, collagenase and the radial elastic properties of arteries

P.B. Dobrin and W.C. Gley

Departments of Surgery and Physiology, Hines Veteran Administration Hospital and Loyola University Medical Center, Hines (Illinois 60141, USA), 9 August 1984

Summary. Studies were performed on 203 pairs of dog carotid arteries subjected to unidirectional radial compression. Treatment with 80 U/ml purified elastase for 90 min decreased radial stress, but treatment with 640 U/ml collagenase for 90 min did not. These data suggest that elastin, but not collagen, contributes to wall resistance to radial compression.
Key words. Elastase; collagenase; radial stress; arterial wall; compression.

Pressurization of arteries causes them to deform in the circumferential, longitudinal and radial directions with little torsion[1]. Deformations in the circumferential and longitudinal directions are tensile, whereas those in the radial direction are compressive. In dog carotid artery, loads in the circumferential direction appear to be borne by both elastin and collagen, while loads in the longitudinal direction appear to be borne almost entirely by elastin[2]. Little is known about the elements that bear compression in the radial direction. Little attention has been given to radial elastic properties because the stress in this direction is only 5–10% of that in the circumferential and longitudinal directions. However, because vascular tissue is essentially incompressible, radial resistance to thinning of the wall can indirectly limit deformations in the remaining directions. The present study was undertaken to assess the effects of enzymatic degradation of elastin and collagen on the uniaxial radial elastic properties of dog carotid artery.

Methods. Segments of relaxed dog carotid artery 4–5 cm in length were opened longitudinally with a sharp scalpel. These tissues then were divided into a pair of vessel segments and were