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Endothelial vesiculo-vacuolar organelles, pockets and multi-layered fenestrated lamellae in the capillaries of the mouse carotid body

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Summary

Fenestrated capillaries represent the basic structural unit in the carotid body. They mediate a characteristic hyperpermeability state in this organ. Endothelial fenestrae and plasmalemmal vesicles are of particular importance in this respect. The present electron microscopic study of the capillaries of the mouse carotid body demonstrates prominent endothelial cell structures that are suggested to be closely related to endothelial fenestrae and plasmalemmal vesicles. These structures include: (1) Vesiculo-vacuolar organelles formed by fusion and intercommunication of vesicles and vacuoles of variable dimensions. (2) Pockets in the form of fenestrated membrane-bound vacuoles that communicate either with the capillary lumen, pericapillary space or both via multiple apertures or fenestrae. (3) Multi-layered fenestrated lamellae where the endothelial cytoplasm is divided into multiple attenuated sheets provided with several fenestrae. The latter two structures are preferentially located in the thick perinuclear region of the endothelial cell. Their fenestrae are always distributed in linear series and show close similarity to the usual chains of fenestrae in the attenuated periphery of the endothelial cells. The individual apertures of the fenestrated vacuoles and multi-layered fenestrated lamellae are closely similar to the stomata of fully opened plasmalemmal vesicles suggesting a relationship between them. Morphological and morphometrical analysis of a series of fenestrae belonging to these structures revealed that they are identical to the usual chains of fenestrae in the attenuated periphery of the endothelial cells.

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Introduction

Blood capillaries are lined by a monolayer of highly specialized endothelial cells. These cells mediate continuous exchange of water and solutes between the circulating blood plasma and the interstitial fluid. For this exchange, endothelial fenestrae and plasmalemmal vesicles are of particular importance. In continuous capillaries, the role of plasmalemmal vesicles in the transcellular passage of substances is now well accepted (Ghitescu and Bendayan, 1992; Predescu and Palade, 1993; Schnitzer et al., 1995; Predescu et al., 1997). In fenestrated endothelia, there exist openings or fenestrae that allow free exchange of materials. In addition, numerous vesicles have also been reported to be involved in transendothelial transport in this type of endothelia (Clementi and Palade, 1969).

Although there is general agreement as to the role of plasmalemmal vesicles in transport across the endothelium, the issue concerning the mechanism(s) underlying this transport is under debate. Some investigators have suggested a shuttling of plasmalemmal vesicles or caveolae between the luminal and abluminal fronts of the endothelial cells (Palade, 1960; Simionescu et al., 1973; Ghitescu and Bendayan, 1992; Bendayan and Rasio, 1996; Rasio and Bendayan, 2002). In this model, the material is endocytosed on one front of the endothelial cell, and the vesicle is then transported towards the other front where its content is discharged by exocytosis. However, internalisation of caveolae has been questioned (Bundgaard, 1983; Bundgaard et al., 1983; Anderson, 1993; van Deurs et al., 1993). These authors have remarked that, in endothelial cells, most, if not all, caveolae connected to the surface are rather stationary and do not detach and move within the cell. On the other hand, according to Parton et al. (1994), plasmalemmal vesicles can lose their connection to the cell surface; a process being regulated by a kinase activity.

Another mechanism involving plasmalemmal vesicles in the process of transport across the endothelium is fusion of such vesicles and possible formation of transendothelial channels connecting the luminal and abluminal fronts of the endothelial cells (Hashimoto, 1972; Bendayan and Rasio, 1996; Rasio and Bendayan, 2002). These channels correspond to a chain of two or more intercommunicating vesicles opening simultaneously on both cell fronts. In addition, fusion and intercommunication between clusters of vesicles and vacuoles with the formation of structures termed vesiculo-vacuolar organelles (VVOs) have also been reported as participating in transcellular transport of the endothelium (Kohn et al., 1992; Dvorak et al., 1996; Feng et al., 1996; Dvorak and Feng, 2001). Opening of the VVOs on the luminal and abluminal surface membrane and creation of transcellular pathways was confirmed by the examination of ultrathin serial sections and computer-assisted-three-dimensional reconstructions (Feng et al., 1996). The VVOs were considered by Kohn et al. (1992), Dvorak et al. (1996) and Feng et al. (1996) to be characteristics of hyperpermeable endothelium of normal venules and tumour associated microvessels although similar structures have also been detected in capillaries of other microvascular beds such as brain (Hashimoto, 1972) and muscle (Simionescu et al., 1975).

In the carotid body microvasculature, VVOs have not been previously reported. The present morphological study was carried out to investigate these organelles and related structures in the fenestrated capillaries of the mouse carotid body. Particular attention was paid to the relationship between these structures and the endothelial fenestrae. In the course of this investigation, the occurrence of a peculiar endothelial structure was noted which we call ''multi-layered fenestrated lamellae'', that appears to be structurally related to VVOs and that has not been described previously in any vascular bed.

Materials and methods

Four male adult 129SvEv x CF1 mice and two adult female FVB mice were sacrificed by inhalation of sevoflurane (Abbott, Wiesbaden, Germany). Immediately upon arrest of respiration, the thorax was opened and a cannula was inserted into the ascending aorta via the left ventricle. The vascular system was flushed with heparin and lidocaine containing rinsing solution (Forssmann et al., 1977) and the animals were fixed by perfusion with 1.5% glutardialdehyde, 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The carotid bifurcations of both sides were dissected, stored for an additional 3 h in the same fixative, and then washed in 0.1 M Tris–HCl buffer, osmicated for 1 h in aqueous 1% OsO_4, washed for 3×15 min in 0.05 M maleate buffer (pH 5.2), stained en bloc for 1 h in 1% uranyl acetate in maleate buffer at pH 6.0, washed again for 3×15 min in 0.05 M maleate buffer (pH 5.2), and then routinely dehydrated in ethanol/propylene oxide and embedded in Epon. Ultrathin sections were cut with an ultramicrotome (Reichert Ultracut E, Bensheim, Germany). The