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Pericyte endothelial gap junctions in human cerebral capillaries

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Summary. Human cerebral tissue has been ultrastructurally studied and gap junctions have been visualized between endothelial cells and pericytes that permit ion exchange. We propose that the functional interrelationship between endothelium and pericytes may play a role in the alteration of capillary diameter for the control of local cerebral blood flow.

Key words: Human cerebral capillaries – Pericytes – Endothelium – Gap junctions

Introduction

Pericytes are macrophage-like cells located on the abluminal surface of cerebral microvasculature. Although their structure and possible function in brain tissue has recently been reported (Cancilla et al. 1972; Le Beux and Willemot 1977, 1978; Schmidley and Wissing 1983), the exact nature of the junctions between the brain pericytes and the endothelial cylinder has been described only recently in the cat caudate nucleus (manuscript in press). Indeed, the cerebral capillaries of the cat caudate nucleus show gap junctions between pericytes and endothelial cells. Such junctions permit ion exchange between pericytes and endothelium. These morphological data lead to the hypothesis of a neurogenic control for cerebral blood flow. Cerebral vessels can respond to a large number of substances including dopamine, serotonin, noradrenalin, acetylcholine, vasoactive peptides and mast cells containing histamine (for review see McDowell and Millikan 1978). In this study, we investigated the junctions between endothelium and pericytes in human cerebral capillaries. Ultrastructural examination of the human blood-brain barrier revealed gap junctions between pericytes and endothelial cells.

Material and methods

Normal human cerebral cortex was removed during surgical procedures from patients with brain tumors. The tissues were prepared for electron microscopy study. After immersion in a fixation solution (1.5% paraformaldehyde-glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2), the specimens were postfixed with ferrocyanide-osmium (Karnovsky 1971), stained with maleate-buffered uranyl acetate and embedded in Epon, with a transitional step in propylene oxide. Regions of cerebral tissue were selected for electron microscopy from 1 μm-thick survey sections stained with toluidine blue. Thin sections of silver-to-grey interference colour were cut with a diamond knife mounted on a LKB ultratome. The sections were stained with uranyl acetate and lead citrate, and investigated in a Philips EM 301 electron microscope.

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Results

The human blood-brain barrier was found to be similar to that of other mammals: endothelial cells, surrounding basal lamina, pericytes and sheet of astrocytic foot processes (Fig. 1). Capillaries were composed of endothelial cells joined at their cytoplasmic margins by junctional complexes and surrounded by a basal lamina. The endothelial cytoplasm contained an elongated nucleus and a few pinocytotic vesicles (Fig. 1). In cross-sections, the capillary cylinder appeared to be surrounded by pericytic processes. At most points on the capillary circumference, the basal lamina divided to enclose the pericytes (Figs. 1, 2 and 3). The basal lamina did not exist in some areas, and the endothelium and pericytic processes were separated from each other by a space 15–17 nm (Figs. 1, 3). In such areas, small regions of specialized membrane apposition (gap junctions) were evident between pericytic processes and endothelial cells (Figs. 2, 3). The seven-layered junctions exist in various contact areas between pericyte and endothelium. In different areas of the capillary cylinder, the pericytic processes invaginate deeply into the endothelium. In such areas, only a tiny portion of endothelial cytoplasm separated the pericyte from the endothelial nucleus (Fig. 3, arrow).

Discussion

Pericytes were initially considered to be a form of smooth muscle cell (Rouget 1873). In the brain, smooth muscle cells are replaced by pericytes at the point where arterioles and venules become a true capillary (Jones 1970). The pericytes of cerebral capillaries are considered to be immature

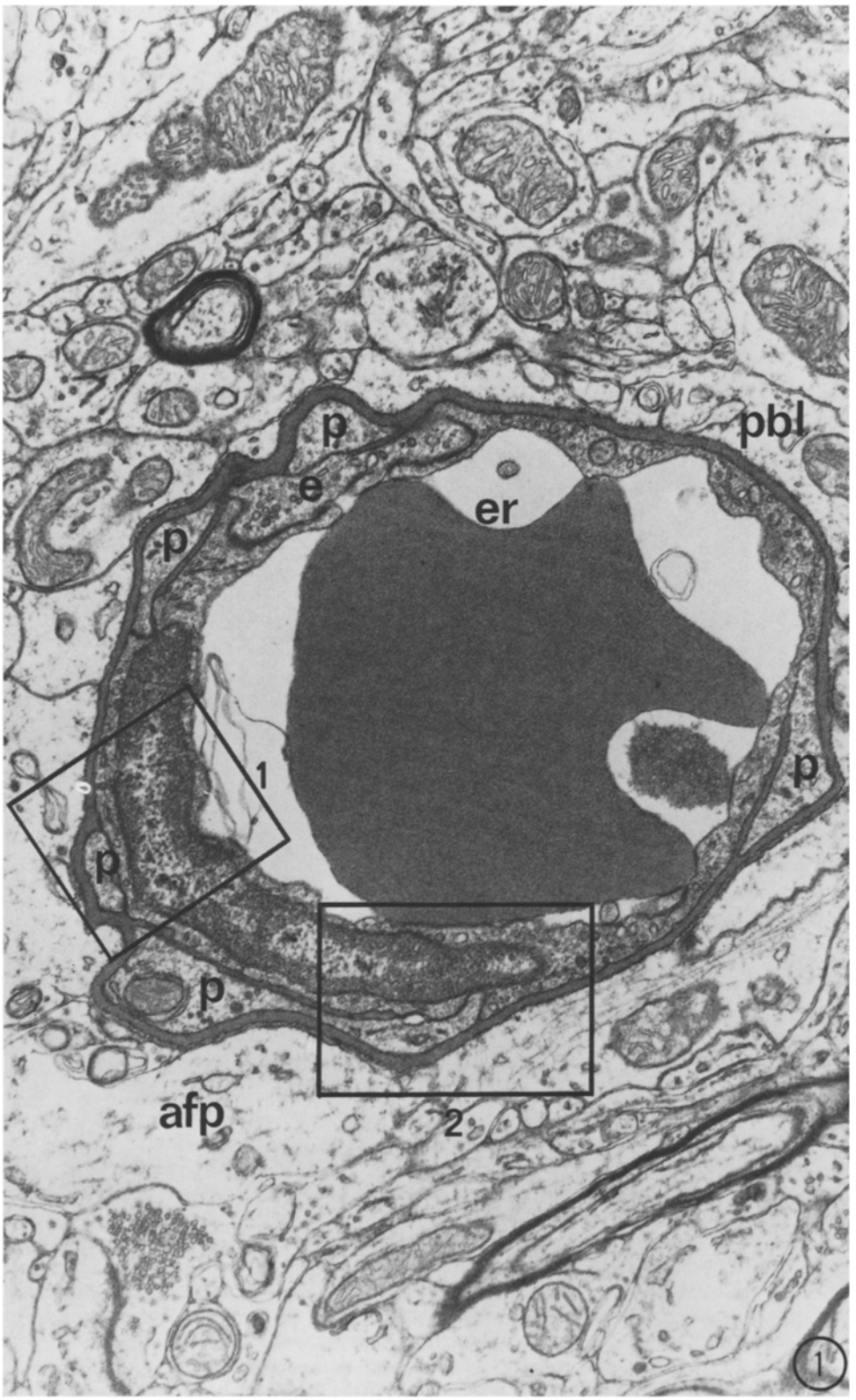
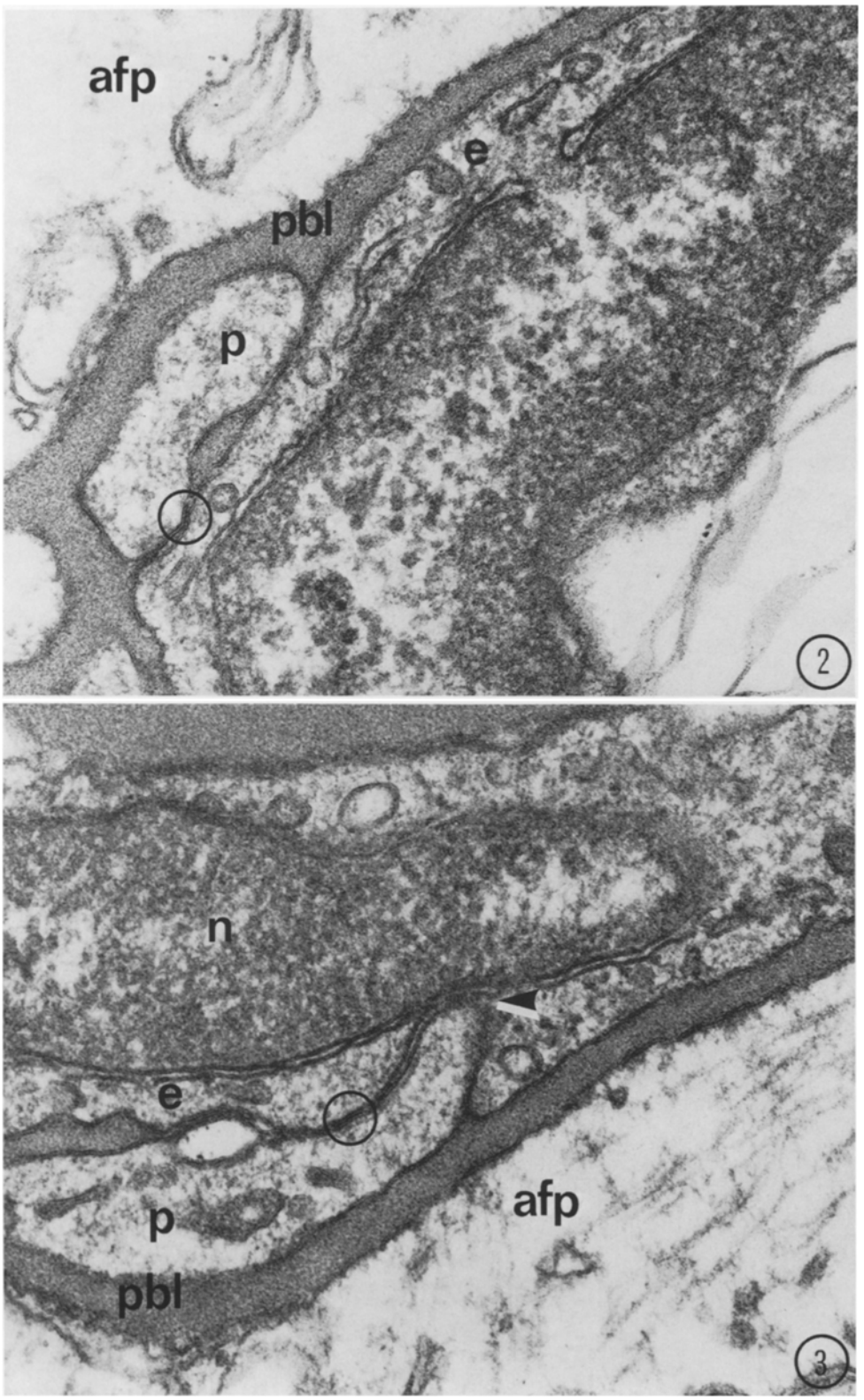


Fig. 1. Cross section of blood-brain barrier; *er*, erythrocyte; *e*, endothelium; *p*, pericytic processes; *pbl*, pericapillary basal lamina. In the neuropil astrocytic foot processes (*afp*), nerve processes and synapses are present. ×23,000

smooth muscle cells (Farquhar and Hartmann 1956). Pericytes occupy a stratum comparable to smooth muscle cells in the medial layer of larger vessels and may perform a similar function: i.e., they may alter the calibre of vessels with which they are associated (Forbes et al. 1977a). To perform this role, pericytes should fulfil at least three morphological criteria (Forbes et al. 1977a): 1) suitably arranged contractile cytoplasmic components; 2) attachments between pericytes and endothelial cells to provide a means of transmitting pericyte contraction to the endothelial cylinder; and 3) components of some system to control pericyte contraction or relaxation (autonomic innervation). The first



Figs. 2 and 3. Represent a magnification of rectangled areas (1 and 2) of Fig. 1. Pericytic processes (*p*) are joined to endothelium (*e*) by a gap junction (circle); *n*, endothelial nucleus; *pbl*, pericapillary basal lamina; *afp*, astrocytic foot process. ×92,000

and third criteria have already been demonstrated (Le Beux and Willemot 1978; Rennels and Nelson 1975). Indeed, the arrangement of cytoplasmic processes in pericytes surrounding capillaries indicated to early investigators that pericytes play a role in capillary contractility with concomitant changes in blood flow (Krogh 1929). Ultrastructural studies have confirmed that pericytes have features of contractile cells (Forbes et al. 1977a; Stensaas 1975; Matsushima and Reiter 1975; Le Beux and Willemot 1977; 1978). Based on biochemical and fine-structural evidence, it has also been suggested that endothelial cells may be contractile (Majno et al. 1969; Rostgaard et al. 1972; Yohro and Burn-

stock 1973; De Bruyn and Cho 1974; Becker and Nachman 1973; Hammersen 1977; Aoki and Tavassoli 1981; Cuevas et al. 1982). Autonomic axon terminals have been described close to capillaries and pericytes (Rennels and Nelson 1975; Forbes et al. 1977b; Wasano and Iijima 1979). Not all pericytes may be innervated, and activity in one cell may be transmitted to contiguous cells in the manner believed to occur between smooth muscle cells in arterial walls (Speden 1979). In order to perform contractile activity, capillary pericytes must possess characteristics similar to smooth muscle cells of larger vessels; that is to say, pericyte-endothelial junctions and interpericyte junctions must exist.

A model of the autonomic neuromuscular junction in blood vessels has been proposed by Burnstock (1975) on the basis of combined electrophysiological, histochemical and electron-microscopical studies. The morphological basis of the model is the appearance of gap junctions between smooth muscle cells; nerves are confined to the adventitial side of the media muscle coat. The deeper smooth muscle cells are responsive to circulating catecholamines.

Although myoendothelial contacts have been detected in different vessels (Rhodin 1967), the exact nature of gap junctions has been defined only recently. Spagnoli et al. (1982) reported gap junctions between smooth muscle cells and endothelial cells in rabbit carotid arteries. Such junctions substantiate the hypothesis of ion exchange (electrotonic coupling) between endothelium and smooth muscle cells (Richardson and Beaulmes 1971). In the neovasa vasorum capillaries of a venous patch grafted into a small vessel, pericytes were joined by gap junctions which were also found between pericytes and endothelial cells (manuscript submitted for publication). Our observations in human cerebral capillaries in which endothelial cells and pericytes were joined by gap junctions agree with the model proposed by Burnstock (1975). The desmosomes and desmosomes-like junctions between pericytes and endothelial cells reported by Forbes et al. (1977a) in mouse heart permit cellular adherence, which is the desmosome's main function (Staehelin and Hull 1978). Based on our data, we propose the following mechanism for regulating microvasculature blood flow in capillaries of rat neovasa vasorum, cat caudate nucleus and human cerebral tissue: 1) capillary endothelial cells and/or their investing pericytes are contractile; 2) endothelial cylinder and pericyte are joined by gap junctions; 3) capillary endothelial cells and/or pericytes receive junctional innervation; when stimulated, they produce endothelial or pericyte contraction, and when blocked they produce relaxation. This hypothesis agrees with the mechanism proposed for the regulation of brain water permeability (Raichle et al. 1976). Our hypothesis agrees with Nakai et al. (1981), when they postulate that the brain capillaries are able to change their diameter and consequently control the cerebral blood flow.

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