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MYOENDOTHELIAL RELATIONSHIPS IN CEREBRAL ARTERIES OF THE HUMAN FETUS

(cerebral circulation/human fetus/electron microscopy)

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Regional close cellular appositions between elements of tunica media and intima (myoendothelial appositions) in cerebral arteries of the human fetus were studied through an electron microscope. Plasmalemmal appositions were observed between endothelial cells and smooth muscle cells in cerebral arteries. The form of these myoendothelial appositions varied from simple flat appositions of plasmalemma and cytoplasmic processes to extensive finger-shaped indentations of endothelial cells onto medial smooth cells. Simple membranous appositions predominated among these cellular appositions of cerebral arteries in the human fetus. The finger-shaped myoendothelial appositions were also observed in the arteries of the surface of the brain. Some of these myoendothelial contacts seem to contain myoendothelial tight junctions (zonula occludens). The myoendothelial appositions may help in the detection and propagation of mechanical(autoregulation) and humoral signals, and may serve as a mechanical support of the fragile cerebral arteries of the human fetus.

Myoendothelial contacts were originally thought to mediate blood-borne humoral signals in the control of peripheral and cerebral arterial tone(1,2). They are now considered to be involved in the myogenic response or arterial smooth muscle cells, autoregulation. However, the possible mechanisms of modulation of vascular tone and the high degree of autoregulation of the cerebral blood flow have been much debated(reviewed by Reis *et al.*, 3.); there is only a few, rather preliminary reports on the myoendothelial relationships in the cerebral blood vessels have been made(1).

In our series of studies on the cerebral blood vessels of the human fetus, ultrastructural observations on myoendothelial relationship in the superficial cerebral arteries have been performed, which have relevance to the discussion of ontogenesis and mechanisms involved in the autoregulation of cerebral blood flow at early stage of the human development.

MATERIALS AND METHODS

Intracranial extracerebral arteries were studied in apparently externally normal, post mortem human fetus(Crown-Rump length: 34 mm). The human fetus was obtained through hysterectomy abortion. The fetus was immersed and fixed in a mixture of 5% glutaraldehyde, 4% paraformaldehyde and 0.2% picric acid(4), 'in toto'. Middle cerebral arteries were excised under a dissection microscope, then post-fixed in 1% phosphate buffered OsO4 (pH 7.2) for 2 hours at 4°C. Tissues were then dehydrated in a series of graded ethanol, and embedded in Epon 812. Thick sections were stained with toluidine blue and examined under a light microscope. After the appropriate area was identified, thin sections were cut perpendicularly to the long axis of the vessels, and mounted on uncoated copper grids. Thin sections, stained with uranyl acetate and lead citrate, were examined and photographed on a JEOL-200CX electron microscope equipped with a tilt stage goniometer.

RESULTS

The wall of the fetal cerebral artery was composed of three layers, intima, media and adventitita. The thickness of the wall was extremely thin. The plump endothelial cells were tightly

held together by junctional complexes, and no fenestrations were observed. The endothelial cells of the fetal cerebral artery were particularly rich in filaments. Most of these filaments have 5 nm in diameter, and scattered within the cytoplasm of endothelial cells. The media was composed of 1 to 3 layers of smooth muscle cells which were contributed to most of the thickness of the arterial wall. The mitotic figures of the medial smooth muscle cells were commonly observed(Fig. 1). The innermost smooth muscle cell along the arterial lumen showed a thin elongated cytoplasm. It was hard to elucidate only from our observations, whether these thin smooth muscle cells were protruded as cytoplasmic processes from the outer smooth muscle cells, or if they were thin elongated smooth muscle cells proper. Lateral membrane-to-membrane appositions between smooth muscle cells(myo-myal appositions) were frequently observed. The coverage of basement membrane on the surface of medial smooth muscle cells was incomplete and discontinued. Numerous collagen fibers were found in the space between cytoplasmic processes of adventitial fibroblasts and medial smooth muscle cells. These collagen fibers possessed regular periodicity of 64 nm intervals.

The most striking morphological evidence of the intracranial artery of the human fetus was the absence of the basement membrane on the abluminal surface of the endothelial cells. In addition to above evidence, the internal elastic lamina had not been developed yet. The space between the abluminal surface of the endothelial cell and adluminal surface of the innermost layer of the medial smooth muscle cells was occupied with some elastic fibers and with amorphous electron lucent materials, and the distance between intima and media was not uniform.

Endothelial cytoplasmic processes frequently broke through the space between endothelial cell and the medial smooth muscle cells to form membranous myoendothelial appositions. Two principal types of myoendothelial appoositions observed, were as follows; flat membranous appositions of cells or cytoplasmic processes, and finger-shaped protrusions associated with indentations of opposite cytoplasm, mostly of endothelial cells were protruded onto medial smooth muscle cells. Typical flat membranous appositions are shown in figures 1,2 and 3. Some of them seem to be equipped with myoendothelial tight junctions (Fig.3). The space between the endothelium and the media is reduced to about 10 nm where those two membranes were closely

Fig. 1. Electron micrograph of cross section of the wall of middle cerebral artery of the human fetus. Luminal surface is lined by single layer of plump endothelial cells and tunica media is composed of three layers of smooth muscle cells(SM). Some of these smooth muscle cells are mitoting. Flat membrane-to-membrane contacts (arrowheads), and finger shaped myoendothelial appositions(encircled areas) between endothelial cells and smooth muscle cells have occurred in several areas. L: lumen of artery, EN: nucleus of endothelial cell, F: processes of fibroblast, Co: collagen fibers. x 18,000

apposed. The individual shape of these flat membranous appositions is remarkably variable, however, the distance along these membranous appositions was varied from 0.5 to 1 micra. In some cases, as the basement membrane was absent on each cell surface, so no particular structure was discernible between the cytoplasmic membranes of endothelial cell and smooth muscle cell.

The close membranous myoendothelial appositions were also observed between the tips of two cytoplasmic processes or between the main body of one cell and the processes of the another (figer-shaped myoendothelial appositions). The majority of finger-shaped appositions are formed by endothelial cytoplasmic processes, which penetrate the space and indent on the adjacent medial smooth muscle cell (Figs. 4,5). The distance between two membranes of endothelial cell and medial smooth muscle cell was about 10 nm, and amorphous electron dense materials were occupied

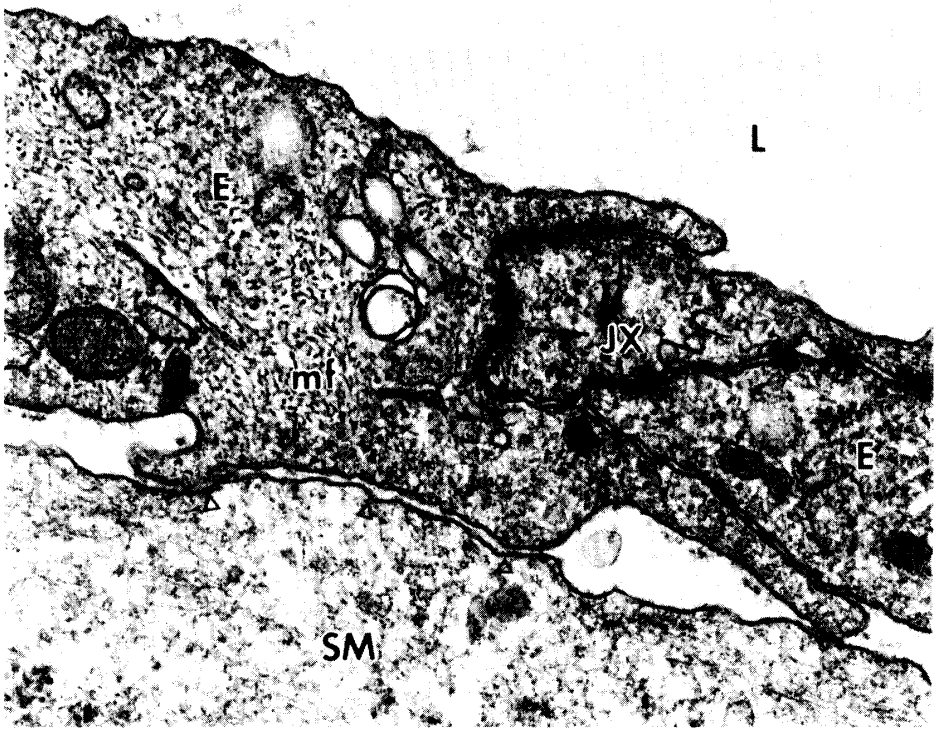

Fig. 2. Each endothelial cell(E) are held together by junctional complexes(JX). Close membrane-to-membrane appositions between endothelial cell(E) and smooth muscle cell(SM) is indicated by the arrowheads, and the distance between them are about 15 nm at their closest apposition. L: lumen of artery. MF: microfilaments. x 80,000

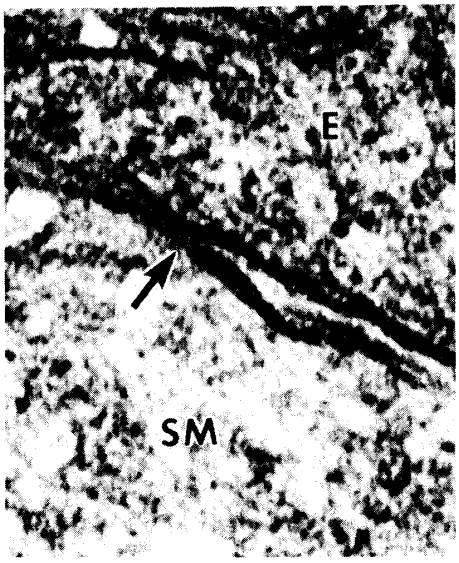

Fig. 3. High magnification electron micrograph, showing close membranous contact between endotheolial and smooth muscle cells(arrow) along the flat membranous apposition of endothelial cell(E) and smooth muscle cell(SM). The outer leaflets of unit membrane are partially fused together. This specimen was tilted 10 degree to show the clear relationship of two membranes.
x 200,000

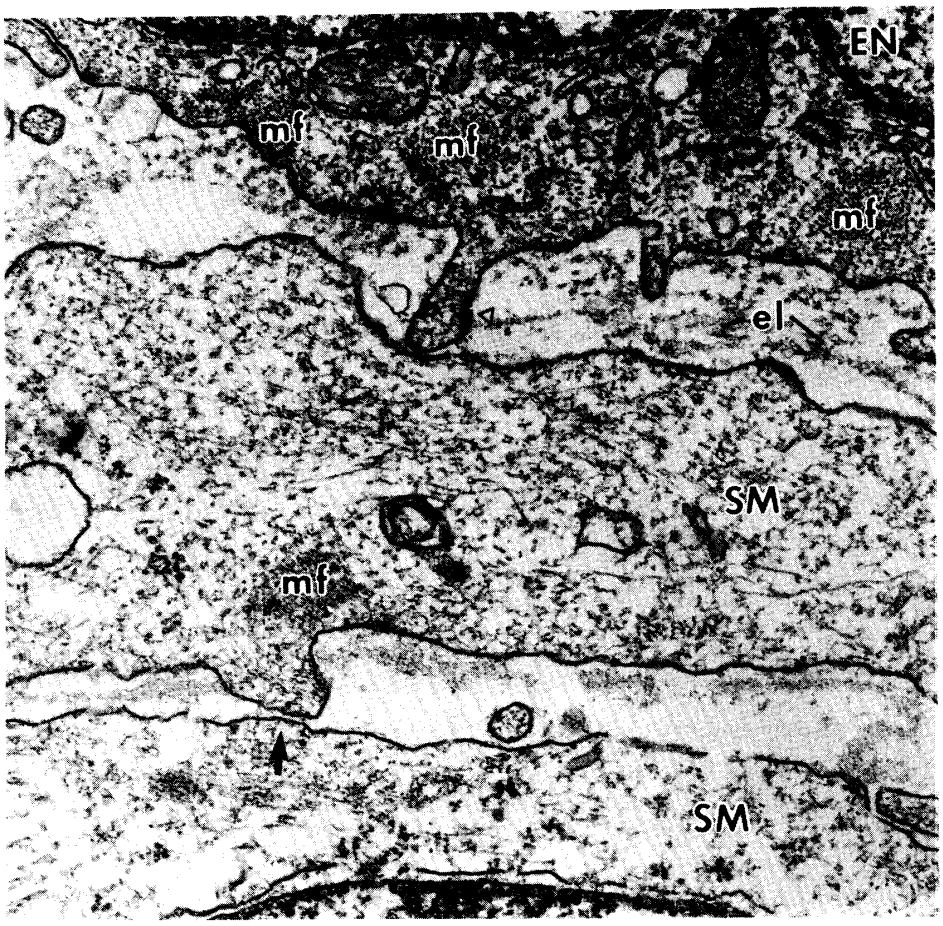

Fig. 4. Finger-shaped cytoplasmic protrusion(arrowhead) is breaking through the space between adluminal surface of smooth muscle cell(SM). The lateral membrane-to-membrane contacts (arrows) between smooth muscle cells in the tunica media is shown in this micrograph. EN: nucleus of endothelial cell, EL: elastic fibers, mf: aggregated microfilaments. x 60,000

within this space (Fig.5).

Finally, as a rare exception, cytoplasmic processes of medial smooth muscle cells were protruded toward endothelial cells, to form myoendothelial contracts. It has not been possible to decide whether each endothelial cell makes such membranous appositions with the medial smooth muscle cells, or whether each cell makes more than one contact. There are bundles of filaments often associated near the roots of cytoplasmic processes, both on the endothelial side and on the smooth muscle side.

Fig. 5. High magnification electron micrograph, showing close membranous appositions(arrowhrads) between tip of finger-shaped processes of endothelial cell, and adluminal surface of smooth muscle. The distance between two apposed membranes is about 10 nm. This specimen was tilted 23 degree to demonstrate the clear relationship of two membrane. E: endothelial cell, SM: smooth muscle cell. x 200,000

DISCUSSION

Myoendothelial contacts have been discovered in the microcirculatory bed of fascia lata of the rabbit and classified as nexus (gap junctions); they were originally thought to be a part of receptor mechanisms for humoral transmitter substances (2). Bevan and Duckels (5) provided evidence to suggest that contraction of the rabbit aorta may be initiated via alpha-adrenergic receptors located on the intimal endothelial cells; the electrical changes which occurred on the endothelial cell membrane were thought to cause changes in smooth muscle tone by spread of excitation. The close relationship between endothelial and smooth muscle cells was taken as evidence for the hypothesis that endothelial and smooth muscle cells act as a coupled system (6,7). Sheridan and Larson (8) concluded that there were myoendothelial and endoendothelial contacts in their general discussion of the structural basis for wide spread junctional (either electronic or metabolic) communication in the peripheral vasculature.

Myoendothelial contacts of the finger-shaped variety, on the other hand, are also discussed in the context of myogenic responses of vascular smooth muscle cells, and referred to the principal mechanism of autoregulation in different vascular beds (9). As recently discussed by Johnson (10), a reduction of vascular calibre with a rise of transmural pressure, as observed in different vascular beds, could be explained by postulating a "sensor (excitable membrane)" coupled in series with the contractile element (11,12,13,14). Since the resulting of vascular contraction will decrease the calibre of the vessel, the increase in transmural pressure would be associated with only a small rise wall tension, making wall tension variable. In the wall of relatively thick arteriole, tension is highest in the endothelium and considerably decreases towards the outer part of the wall. The myoendothelial membranous appositions discovered by Rhodin (2), are thus localized at sites of stress concentration. This led to the suggestion that the close membranous myoendothelial appositions are the morphological basis for the postulated "tension sensor" (13).

To our knowledge, there have been only a limited number of reports on plasmalemmal contacts between the intima and media of intracranial arteries (1,15). In our present study, close membranous myoendothelial apposotions have been observed in the intracranial extracerebral arteries of early stage of the human fetus. Some of these close membranous appositions which seem to be equipped with focal tight junctions (zonula occuludens, 16), representing the structural basis of the myoendothelial adhesion, in the cerebral arteries of the human fetus. According to recent investigation, the myoendothelial contacts in the microcirculatory bed are supplemented by endo-endothelial and myo-myal contacts. This evidence is supporting the general view of wide spread junctional electronic and metabolic cooperation in the peripheral vasculature (1,8,17). There are, however, discrepancies in the incidence and morphology of the membrance cotacts between the superficial and intraparenchymal cerebral arteries; this might be a reflection of functional significance (1,15). Flat membranous appositions equipped with tight junctions may serve to transmit humoral signals (mediated by the endothelial cells) from the lumen of the vessels to smooth muscle cells (18,19). If it was accepted that the myogenic response is restricted to the intracerebral arterioles, the finger-shaped

type of myoendothelial contacts, which have the overall distribution along the arterial wall, may therefore the morphological basis from which myogenic signals spread. The protruding part of the finger-shaped appositions supposed to be as the "tension sensor", which initiates smooth muscle depolarization when stretched by an increased transmural pressure.

In the present study, membranous appositions between smooth muscle cells and endothelial cells, also frequent lateral membrane-to-membrane appositions between smooth muscle cells have been demonstrated. The significance of these membranous apposition is at present unknown. Since communicating junctions occupy only part of the membranous appositions and some junctions might have escaped indentations due to non-perpenduicular sectioning of the involved membranes. It seemed reasonable to assume that tight junctions are standard attribute of the myoendothelial contacts in fetal cerebral vessesls. However, the possibility exists that such contacts are related to mechanisms controlling the tone of the vessels, or they might be one of the protecting apparatus for the fragile fetal vascular endothelial cells. Middle cerebral artery of the early human fetus is deprived of innervation. Present investigation indicates that while the adult cerebral vessels may be subjected to both neural and chemical control (3), the tone of the fetal cerebral artery might be regulated mainly by chemical stimuli, most likely mediated through the myoendothelial contacts.

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