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Ribosome-associated Membrane Contacts Between Astrocytes in the Anoxic Brain

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Summary. Between adjacent astrocytes in cerebral and particularly cerebellar cortices of brains unsuccessfully perfused or subjected to 2–30 min anoxia or short (5 h) autolysis, a new distinct and unusual variant of an intercellular apposition was observed, which derived from already pre-existing membrane contacts. This membrane apposition was characterized by thicker contacting plasma membranes than usual, narrower intercellular distance, a fine para- and intermembranous material and ribosomes in the paramembranous position. In addition to these ribosome-associated membrane appositions ribosomes-associated gap junction and microtubule-associated junction were observed in isolated cases. The reason for binding of ribosomes and microtubules to the plasma membrane and their function in this site is unknown.

Key words: Plasma membrane – Intercellular junctions – Ribosomes – Astrocytes – Anoxia

Introduction

Protoplasmic astrocytes have a very complicated surface and their numerous irregular processes frequently come into direct contact with one another. Plasma membranes of adjacent astrocyte processes are mostly separated by a distance of 15–20 nm. Specialized membrane contacts of the zonula adhaerens and gap junction type also occur which can be of particularly large extent in the cerebellar cortex between the Bergmann fibers sharing a long straight interface (Palay and Chan-Palay 1974; Peters et al. 1976).

In addition to these typical membrane junctions, another type of membrane contact characterized by an association with ribosomes was observed in our material subjected to anoxia.

The present communication aims at reporting the results of an ultrastructural analysis of this ribosome-associated contact (RAC), comparing it with some similar rare observations presented in other studies and discussing a possible mechanism of its origin.

Material and Methods

Adult albino mice, rats and rabbits were anesthetized with chloral hydrate, ether or pentobarbital and perfused through the heart with a 1% glutaraldehyde/1% paraformaldehyde mixture (Palay and Chan-Palay 1974). Artificial respiration with oxygen before perfusion was applied in some animals, perfusion after a shorter than 1 min apnoeic pause or delayed perfusion after a 2–30 min apnoeic pause in others. The human brain tissue was immersed in the same mixture 15 min after its withdrawal during an operation and 5 h post exitum. The tissue blocks from the cerebral and cerebellar cortices were post-fixed with phosphate-buffered 2% osmium tetroxide, stained with uranyl acetate, dehydrated in ethanol, passed through propylene oxide or acetone, and embedded in an Epon-Durcupan mixture. Several tissue blocks were fixed with Na-cacodylate-buffered 1% glutaraldehyde containing 0.5% ruthenium red and post-fixed with 2% osmium tetroxide in the same buffer containing 0.5% ruthenium red. Blocks were dehydrated and embedded in the same way as the others. Ultrathin sections were stained with uranyl acetate and lead citrate and viewed in a Tesla BS 500 electron microscope.

Results

Between adjacent perivascular astrocyte processes in the human cerebral and cerebellar cortices and particularly between the adjacent Bergmann fibers in the cerebellar cortex of laboratory animals, a new distinct intercellular contact was found as early as 2 min after anoxia began. It differed in several aspects from the normally occurring simple membrane apposition, zonula adhaerens, and gap junction.

There are several reasons why the contact was so conspicuous and, in tis fully differentiated form, easy to find: (1) the contacting plasma membranes became thicker and electron-denser, (2) the intercellular dis-

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tance became narrower, (3) fine paramembranous and intermembranous material appeared, and (4) ribosomes appeared in the paramembranous position.

The first ultrastructural changes which lead to the formation of the RAC appeared as early as the first minute of anoxia. The inner lamina of the plasma membrane became denser and thicker in some places (Figs. 1, 2) expanding as much as twice the original thickness (Fig. 3). An increased osmiophilia was observed also on the outer membranes of some mitochondria (Figs. 1, 5). The distance between the plasma membranes of the adjacent astrocyte processes, being originally about 15 nm in our material, gradually decreased to 8–10 nm simultaneously with the appearance of the semi-dense material in the intercellular gap. This material was difficult to distinguish, but in its most differentiated form it seemed to bridge the gap as fine septae or globular particles, about 8 nm in diameter, in places with a pseudoperidoicity of 12–16 nm (Figs. 1, 3, 5). Thus, the contact was reminiscent, at some places, of a septate-like junction, or rarely, of a gap junction. However, ruthenium red staining did not reveal any gap subunits (Fig. 3, inset).

At the cytoplasmic faces of the plasma membranes with increased osmiophilia, rosettes or clusters of dense particles appeared (Figs. 2, 4) which later dissociated and aligned closely along the plasma membrane (Fig. 5). Their diameter (about 20 nm) which is identical with that of ribosomes associated with the granular endoplasmic reticulum in the same cells, and a content of smaller and larger subunits in some of them allowed one to identify them as ribosomes. The linear distance between individual ribosomes was sometimes smaller than their diameter and there was apparent an approximately hexagonal array in some sections parallel to the plasma membrane (Fig. 9). In several places, a very thin filament was found to bridge the distance between the neighbouring ribosomes which possibly could represent the messenger-RNA (Figs. 8, 10). Neurofilaments and microtubules were sometimes found in juxtaposition to the RAC (Figs. 8, 10).

Some findings indicated that plasma membrane and paramembranous changes appeared originally in foci and then gradually enlarged their extent (Figs. 1, 2). The shortest RAC in our material approached 0.5 µm, the longest 8 µm. The gap junction was frequently found in the immediate neighborhood of the RAC (Fig. 12).

It was possible to find a small amount of semi-dense granulofilamentous material between ribosomes in some places. In the human autolytic material its density and extent were greater and ribosomes, at first still quite distinct (Fig. 10), disappeared completely at the end (Fig. 11).

Another feature of the RAC was an occasional occurrence of four plasma membranes in a tandem (Figs. 7, 8). The central couple of membranes belonged to a very attenuated astrocyte process, an arrangement never found in normal cerebellum. Only two external plasma membranes were associated with ribosomes in such a case.

Our previous experiments included material from the rat thalamus and the rabbit cerebellar cortex perfused after a very short (about 30–60 s) period of anoxia. By chance, a ribosome-associated gap junction (Fig. 13) and a septate-like or gap junction-like contact, associated with a parallel array of microtubules were found between the adjacent astrocyte processes in such tissue (Fig. 14).

The ultrastructure of the RAC is summarized in the diagram (Fig. 15).

Discussion

A typical gap junction associated with ribosomes after autolysis was found by David-Ferreira and David-Ferreira (1973) in the rat liver. According to these authors, an influx of Ca^{2+} and other divalent cations into cells and their binding to the junctional membrane during autolysis could be responsible for binding of ribosomes to the same site. Schuster and Hajós (1978) found junctions identical with RACs observed in our material, and they considered them not to be artificial but normal structures belonging "to the class of gap junctions". They did not consider the electron-dense particles localized along the inner faces of apposed plasma membranes to be ribosomes but they speculated that these particles represented "morphological landmarks" of ion passage through the junction.

There exist structural differences between gap junctions expressed mainly by variations in the width of the gap and the prominence and degree of organization of the lattice of connexons (Brightman and Reese 1969; Bennet and Goodenough 1978). Their permeability is influenced by the concentration of calcium ions within the cell and an increase in intracellular Ca^{2+} triggers the uncoupling of cells. Gap junctions can be also newly formed or reversibly disappear under various experimental conditions or functional states of cells (e.g., Johnson et al. 1974; Bennet and Goodenough 1978; Kannan and Daniel 1978; Yee and Ravel 1978). However, contrary to Schuster and Hajós (1978), we do not consider RAC to be gap junction.

According to Palay and Chan-Palay (1974), the zonula adhaerens-resembling junction between normal astrocyte processes in the cerebellar cortex is marked by densities adherent to the cytoplasmic surfaces of the apposed plasmalemmas and filling up the interstice

between them. One can say that RACs, as described in our report, partly resembled a zonula adhaerens in some phases of their differentiation. However, whereas a regular zonula adhaerens between epithelial cells has a 15–25 nm wide interspace, usually filled with very fine filamentous material (Staehelin 1974), the interspace in our RAC is only about 8–10 nm wide, filled with bridging subunits and thus rather similar to a septate junction.

The membrane apposition described in our report is neither a typical intermediate junction (zonula adhaerens) nor a typical septate or gap junction. It seems unlikely that it might represent a gap junction in a state of splitting (unzippering) because according to our personal observations and to David-Ferreira and David-Ferreira (1973) gap junctions do not disappear either after short anoxia or after several hours of autolysis. In addition to this, gap junctions of such an extent as RACs were not observed in the normal brain tissue at places where in the anoxic brain RACs were formed. Therefore, we suggest that the RAC between cerebellar cortical astrocytes represents a quite unusual variant of an intercellular apposition which derived from an already pre-existing membrane contact between astrocytes during anoxia.

According to Karlsson et al. (1975), potassium ions induce increased osmiophilia and formation of granular deposits at the extracellular side of the plasma membrane and sodium ions induce a widening of the intracellular side of the plasma membrane. The mechanism of ion-dependent osmiophilia is obscure. Peracchia and Robertson (1971) observed increased osmiophilia of the axonal plasma membrane, outer mitochondrial membrane and endoplasmic reticulum of crayfish after electrical stimulation, asphyxia, or treatment with reducing agents. According to them, asphyxia can be associated with changes in the oxidation-reduction potential. These changes result in a reduction of disulfide bonds and thus in an unmasking of SH groups which are very reactive with osmium. We suppose that the increased osmiophilia of the plasma membranes in the RAC can be explained in the same way but it is not clear why it is so enhanced just between the astrocytes.

Monoparticulate glycogen β-particles, having 15–30 nm in diameter, are difficult to distinguish from ribosomes which are usually about 15 nm in diameter, but may reach as much as 30 nm (David 1978). A comparison of ultrathin sections – one stained with uranyl acetate alone, the other with lead citrate – helped to resolve this difficulty, for ribosomes were visualized in sections stained both with uranium and lead whereas glycogen should be evident only in the lead-stained sections (Ghadially 1977).

Fig. 1. Plasma membrane contact between two astrocyte processes after very short anoxia (about 30 s to 1 min). Note increased osmiophilia of the inner layer of the plasma membrane and the outer mitochondrial membrane. The intercellular material is also apparent. Mouse cerebellar cortex. Scale bar = 200 nm

Fig. 2. The intercellular gap is narrowed at the sites where foci of the RAC are formed (*arrows*). *ger* granular endoplasmic reticulum. Mouse cerebellar cortex, 20 min anoxia. Scale bar = 100 nm

Fig. 3. The RAC with the apparent intercellular (*arrows*) and paramembranous semi-dense material. The ribosomes are also apparent. Mouse cerebellar cortex, 20 min anoxia. *Inset:* ruthenium red staining. Scale bar = 100 nm

Fig. 4. The RAC with clustered and aligned ribosomes. Mouse cerebellar cortex, 20 min anoxia. Scale bar = 100 nm

Fig. 5. The RAC with aligned ribosomes. A septate-like appearance (*arrow*) is apparent in some places. Osmiophilia of the outer mitochondrial membrane is increased (*empty arrowhead*). Mouse cerebellar cortex, 20 min anoxia. Scale bar = 100 nm

Fig. 6. The RAC of a large extent. Mouse cerebellar cortex, 20 min anoxia. Scale bar = 0.5 µm

Fig. 7. The double RAC, partially obliquely sectioned. An interposed astrocyte process (*asterisk*) is severely attenuated. Mouse cerebellar cortex, 20 min anoxia. Scale bar = 250 nm

Fig. 8. The double RAC. The central couple of membranes belongs to a very attenuated astrocyte process. A thin filament bridging two ribosomes (*arrow*) may represent the messenger – RNA. *f* neurofilament. Mouse cerebellar cortex, 20 min anoxia. Scale bar = 200 nm

Fig. 9. The section parallel to the RAC. An approximately hexagonal arrangement of ribosomes (*circled*) is apparent. Mouse cerebellar cortex, 20 min anoxia. Scale bar = 100 nm

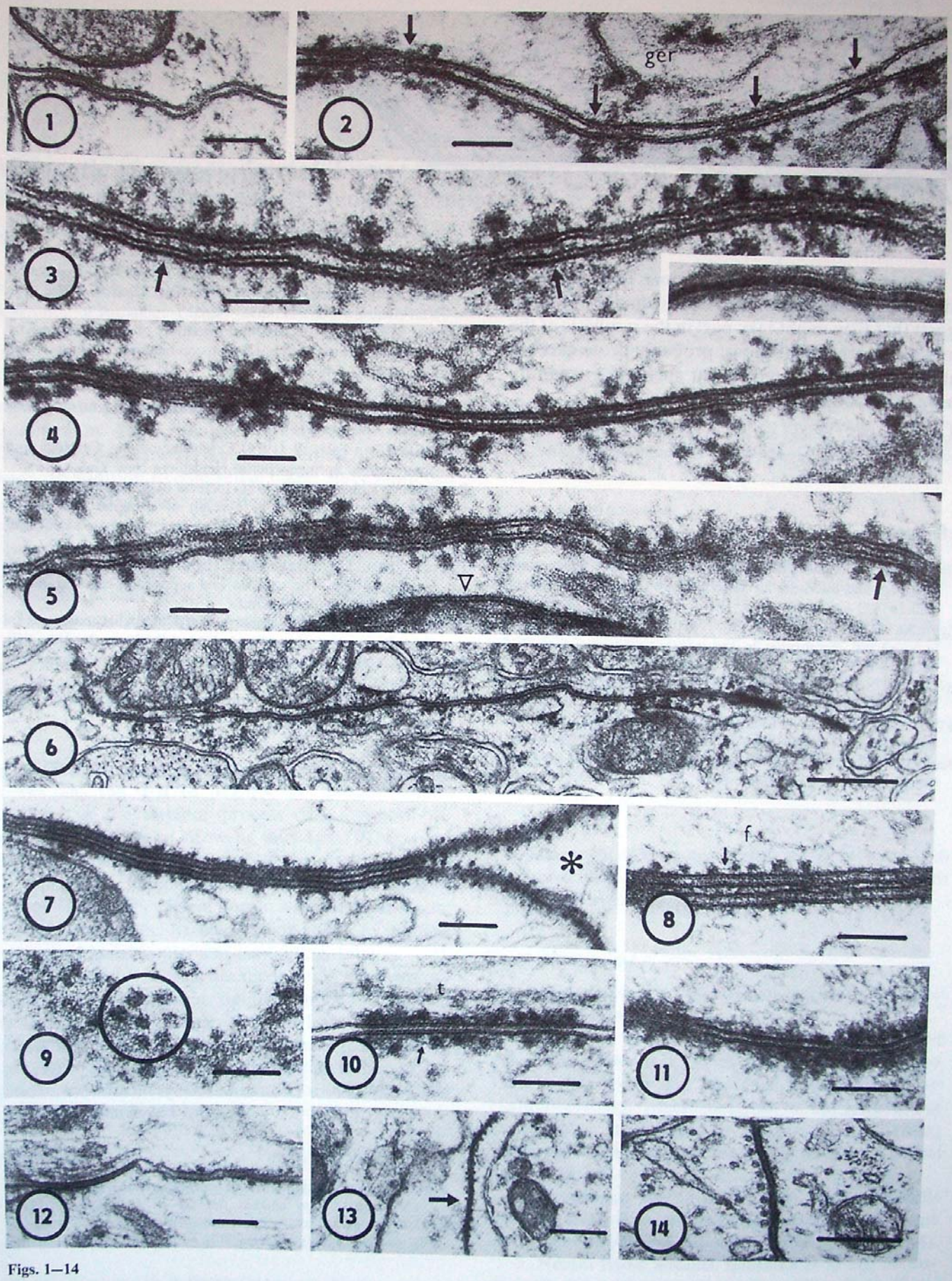
Fig. 10. The RAC with distinct paramembranous density. A possible messenger-RNA filament is marked by an arrow. *t* microtubule. Human cerebellar cortex, 5 h post exitum. Scale bar = 200 nm

Fig. 11. The RAC after 5 h autolysis. Human cerebellar cortex. Scale bar = 200 nm

Fig. 12. Gap junction in a close relationship with the RAC. Mouse cerebellar cortex, 20 min anoxia. Scale bar = 200 nm

Fig. 13. A ribosome-associated gap junction between astrocyte processes (*arrow*). Rat thalamus, 30–60 s anoxia. Scale bar = 0.4 µm

Fig. 14. One of the serial sections of a microtubule-associated membrane contact between astrocyte processes. Rabbit cerebellum, 30–60 s anoxia. Scale bar = 200 nm



Figs. 1–14

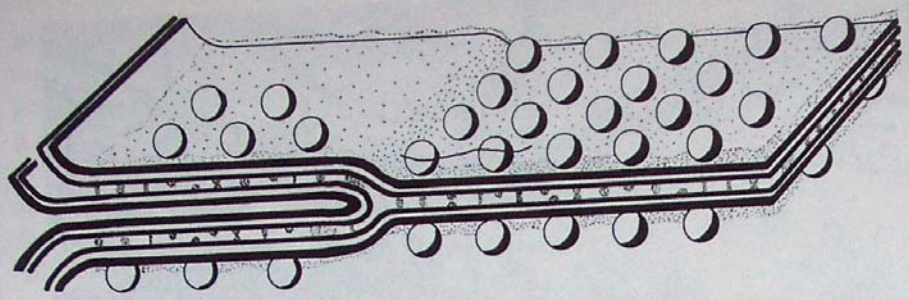


Fig. 15. Diagrammatic representation of the RAC

The reason for binding of ribosomes to the junction and their function in this site is unknown. However, we suggest that they might take part in the synthesis of the semi-dense paramembranous, probably proteinaceous material which occurred in an increasing quantity during differentiation of the RAC.

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