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Gap-like junctions between neurons and glia in the superior colliculus of mammals

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Summary. The neuron cell bodies and glia cells are separated by a narrow intercellular cleft of 200 Å. We present here evidence that perikarya neuronal and perineuronal glia of the superior colliculus of the *Oryctolagus Cuniculus* communicate through gap-like junctions according to ultrastructural parameters. These junctions would mean some degree of electronic coupling between neuron cell bodies and perineuronal glia cells.

Key words: Gap-like junctions, Glia, Superior colliculus

Introduction

Glial cells have many hypothesized roles in the nervous system, but ion and volume regulation is now one of the best documented. The extracellular K^+ regulatory mechanisms are particularly interesting. K^+ can be removed from a site of accumulation, such as around active neurons, by extracellular diffusion, intracellular net uptake along with anions or in exchange for cations, and passive entry via glial K^+ channels, with redistribution to areas of low K^+ concentration, forming the «spatial buffer mechanism» (Abbot, 1986). It has been suggested that the K^+ incorporation by astrocytes is a homeostatic mechanism posterior to nervous conduction. The neuron-glia interactions have been studied by Grossman and Seregin (1977) and by Latzkovitz (1974); the K^+ carriage between glial and neuronal cells has been demonstrated in several different astrocytic culture systems. A comparison of the uptake capabilities of astrocyte and neuron cultures suggest that the glia in vivo could be responsible for a greater proportion of K^+ uptake than neurons. The cellular cultures showed the presence of «high potassium glia», whose function was the uptake of the excess in the surrounding neuronal space. As a subsequent phenomenon to the neuronal activity, the K^+ flow in the neuron-glia intercellular space depolarizes the glial membrane. The depolarization is passively propagated through the glial cells that communicate by gap-like junctions (Massa and Mugnami, 1982; Mugnaini, 1982). This fact helps to regulate the extracellular K^+ concentration, but also unleashes glial metabolic responses, such as glicogen synthesis and its neuronal carriage. Intercellular contact zones between connected cells has suggested that gap-like junctions act like low resistance ways, that make the intercellular ionic carriage easier.

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Materials and methods

The experimentation animal was the pigmented rabbit (*Oryctolagus Cuniculus*). Following anaesthetization (6% chloral hydrate), adult male (30 postnatal days) were perfused through the left ventricle with saline solution followed by fixative (2.5% paraformaldehyde, 2% glutaraldehyde in 0.1 M Sörensen phosphate buffer with 0.5% Cl_2Ca).

Subsequently, the brains were removed and fixed by immersion in the same fixative. Brains were sectioned in sagittal planes, then osmicated (2% $Os0_4$), embedded and sectioned for electron microscopy. Ultrathin sections, counterstained with lead citrate, were examinated with a Philips-301 electron microscope. Photographs were taken on Kodak 4489 E.M. film.

Results

Transmission electron microscopy mostly revealed the presence of a clear intercellular space between the limiting membranes of the neuronal perikarya and the perineuronal glia; this space was about 200 Å and both membranes ran parallel to each other (Fig. 1). They showed the classical morphology of membranes in thin sections.

In a small number of cases, we found images that suggested the presence of gap-like junctions. We observed between neuron perikarya and perineuronal glia, the existence of zones occupied by electrondense material, and proximity of both membranes with a reduction of the intercellular space around 30-40 Å. These ultrastructural characteristics agree with the classical description of the gap-like junctions. The membrane systems of neuron perikarya were poor in organelles and only contained some endoplasmic reticulum cisterns, that stood close to those membrane zones although not in direct apposition (Figs. 2, 3).

Fig. 4 shows similar images, where the previously described submembranal cisterns could be seen. The observation of the perineuronal glia that formed gap-like junctions did not reveal any organules in the reduced cytoplasmic fraction.

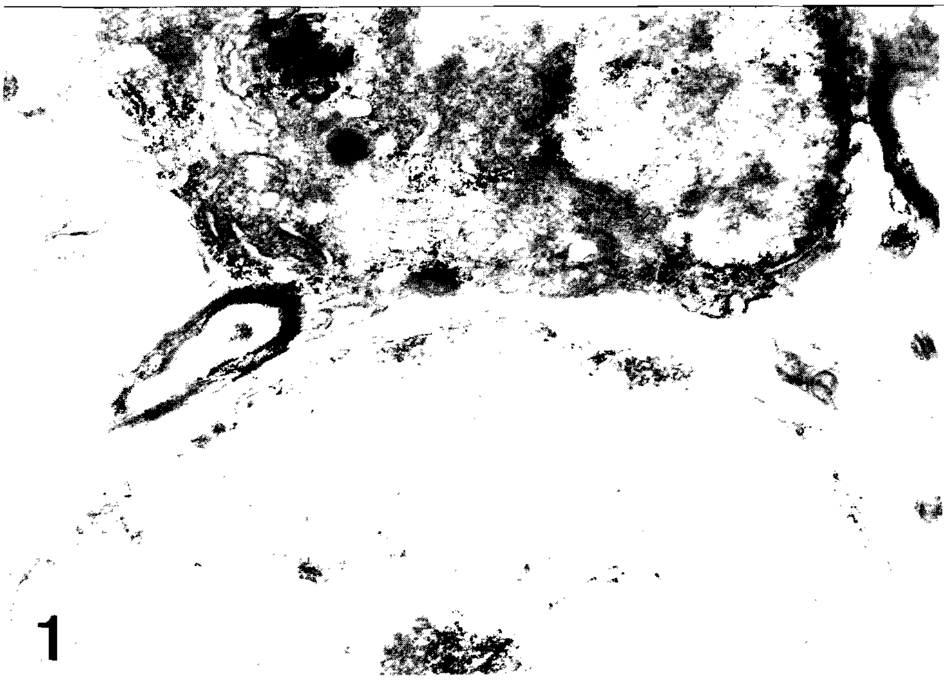

Fig. 1. Intercellular cleft between neuronal perikarya and perineuronal glia. × 6000

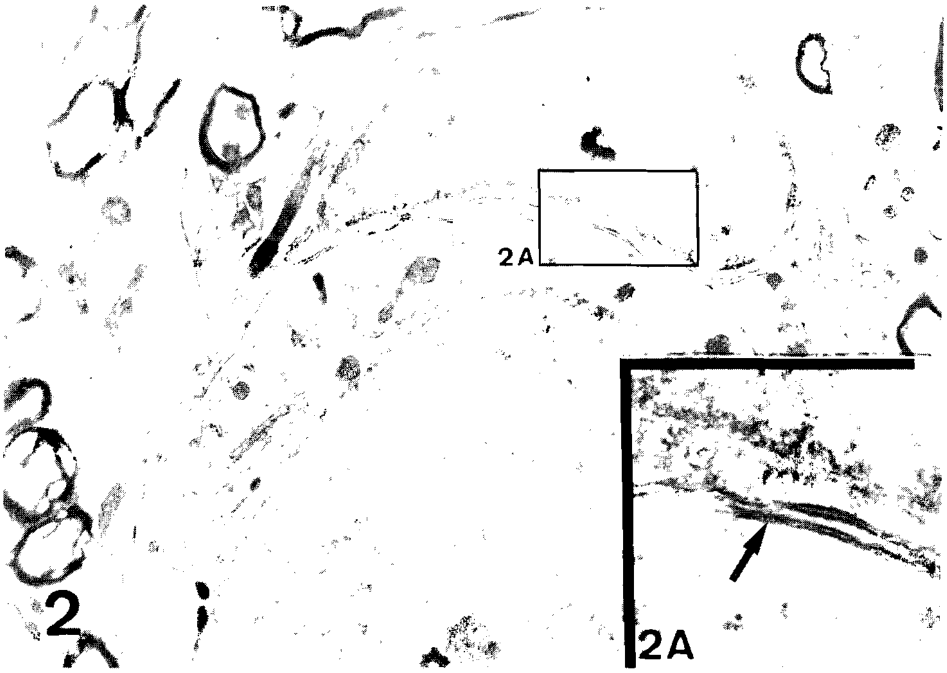

Fig. 2. Gap-like junctions. The membrane system of neuron perikarya contains only some endoplasmic reticulum. Intercellular space around 30-40 Å. × 5000

Fig. 2A. The arrow shows a detail of gap-like junction. × 25000

Fig. 3. Gap-like junctions between neuronal perikarya and perineuronal glia. × 5000

Fig. 3A. The arrow shows a detail of cisterns of endoplasmic reticulum. × 25000

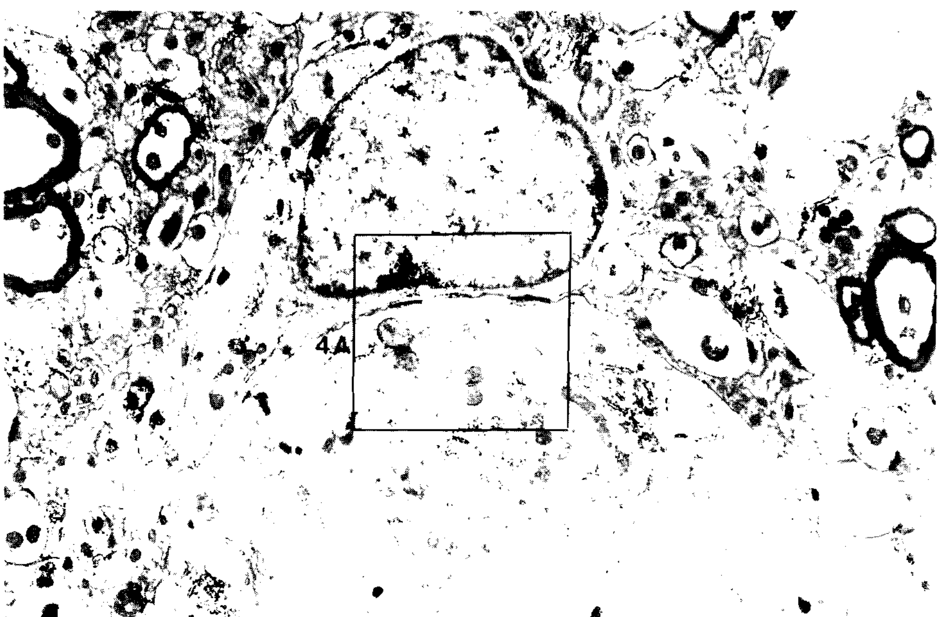

Fig. 4. Heterocellular gap-like junction between a neuron perikarya and a glial cell. × 4500

Discussion

The gap-like junctions have been described between adjacent glial cells in the central nervous system. These junctions are more frequent in the cellular processes related to the limiting glia or between the glia that ensheat the blood vessels. The gap-like junctions allow the free interchange of ions and small molecules, pointing out the fact that astrocytes form a brain syncitio-like compartment (Bennet, 1978; Varon, 1979). By

Fig. 4A. Frame of Fig. 4, reduction of the intercellular space, electrodense material. × 19000

means of quantitative freeze-fracture replica examination two kinds of particles are described. One consists of large, irregular particles densely packed; these are called polygonal particle junctions and are present just below the cerebellar surface in the processes of the glia limitants, between radial Bergman fibres, and occasionally throughout the molecular layer. The others are called assemblies; these are small intramembrane particles (6-7 nm periodicity) packed in orthogonal arrays in square or rectangular aggregates. Their localization in astrocytic membranes juxtaposed to vascular structures suggests a role in transport mechanisms (Gabella, 1981; Massa and Mugnami, 1982; Duffi, 1983).

There are few references about the presence of gap-like junctions between neuronal and non-neuronal elements (Lane, 1981). In this study we describe membrane specializations between neurons and glial cells in the superior colliculus of mammals, and we identify them as gap-like junctions according to their ultrastructural characteristics. These same kinds of intercellular junctions have been localizated in the teleosteo Procambarus (Cuadras et al., 1985). These authors describe gap-like junctions between neurons and glial cells in abdominal ganglios of those animals. Contrasting with our observations, they found a large number of membrane specializations along the limiting membranes of both kinds of cells. Actually it is supposed that gap-like junctions could signify a swift ionic diffusion way, specially for K^+ carriage in both directions. This fact would mean some kind of electronic coupling for the cited cells.

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