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Immunohistochemical localization of glial fibrillary acidic protein (GFAP) in rat pineal stalk astrocytes

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Summary. In the present work, the presence and distribution of astrocytes in the rat pineal stalk is investigated applying an immunohistochemical technique for the demonstration of glial fibrillary acidic protein (GFAP) on Epon-embedded semithin sections (0.5 μm thick). GFAP-immunorective cells are evenly and regularly distributed along the entire pineal stalk. The GFAP-immunoreactive cells display a stellate shape showing variable numbers of cell processes that are mainly oriented parallel to the longitudinal stalk axis. Astrocytic processes show a clear tendency to encircle the remaining elements of the pineal stalk; i.e., pinealocytes, nerve fibres and blood vessels. Furthermore, glial processes form a cover layer separating the stalk from surrounding anatomical structures.

Key words: Glial fibrillary acidic protein, Astrocytes, Pineal gland, Pineal stalk, Rat

Introduction

The Wistar rat pineal stalk is a thin cord that connects the roof of the third ventricle to the pineal gland. The pineal stalk contains pinealocytes, nerve fibres and glial cells (Luo et al., 1984a,b; Calvo and Boya, 1985). The presence of occasional oligodendrocytes and Schwann cells has also been described by Luo et al. (1984a).

Nerve fibres are the main component of the pineal stalk both in number and physiological importance. Most of these fibres are unmyelinated axons with different postulated origins. Sympathetic fibres that reach the brain through the pineal stalk have been described (Bjorklund et al., 1972; Wiklund, 1974). However, the major evidence supports the view that the main nervous component of the pineal stalk are central fibres (commisural or central pinealopetal fibres) that arise in the habenular nuclei and the stria medullaris. In rodents, this neural component has been extensively investigated by using electrophysiological methods (Semm et al., 1981; Reuss et al., 1984), electron microscopy (Schneider et al., 1981; Moller and Korf, 1983a) and applying anterograde (Reuss and Moller, 1986) or retrograde (Guerillot et al., 1982; Dafny, 1983; Moller and Korf, 1983b) transport of horseradish peroxidase. Immunohistochemical studies have demonstrated peptidergic nerve fibres as well (Buijs and Pevet, 1980; Nürnberger and Korf, 1981; Ronnekleiv and Kelly, 1984; Schon et al., 1985; Moller et al., 1990).

The presence of glial fibrillary acidic protein (GFAP)-immunoreactive cells in the rat pineal gland has been clearly shown (Moller et al., 1978; Schachner et al., 1984; Zang et al., 1985; Calvo et al., 1988a). These cells are sparse and always located in the proximal third of the gland. Although glial cells have been noted in the pineal stalk at the ultrastructural level (Luo et al., 1984a; Calvo and Boya, 1985), no immunohistochemical studies on these cells have been reported so far.

The aim of the present work was to study the presence, distribution and morphology of this cell type of the rat pineal stalk applying immunohistochemical techniques for GFAP on semithin sections.

Materials and methods

Twelve adult Wistar rats of both sexes kept under routine laboratory conditions (14L:10D, water and food ad libitum) were decapitated under ether anaesthesia. The brains and the pineal glands in situ were fixed by immersion in 2% glutaraldehyde - 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 at 4° C. The cerebral hemispheres were dissected in order to allow quick fixation of the pineal stalk. After fixation, a block was cut with the pineal complex resting on the corpora quadrigemina and embedded in Epon without previous osmication. Blocks were properly oriented and longitudinal and transverse sections of the pineal stalk

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were obtained. Semithin sections (0.5 μm thickness) were cut with an LKB ultramicrotome. Sections were etched for 20 minutes with sodium ethoxide, incubated in non-immune swine serum and immunostained for GFAP. A peroxidase-antiperoxidase (PAP) technique (Taylor, 1986) using a polyclonal rabbit anti-bovine GFAP antiserum (Dakopatts, Denmark) at 1:300 dilution was applied.

Results

GFAP-positive cells displaying characteristic astrocytic profiles were clearly shown in the pineal stalk (Fig. 1). The strong positivity of the glia limitants of the adjacent cerebral tissue, constituted an intrinsic positive control (Fig. 6).

GFAP-immunoreactive elements were conspicuous

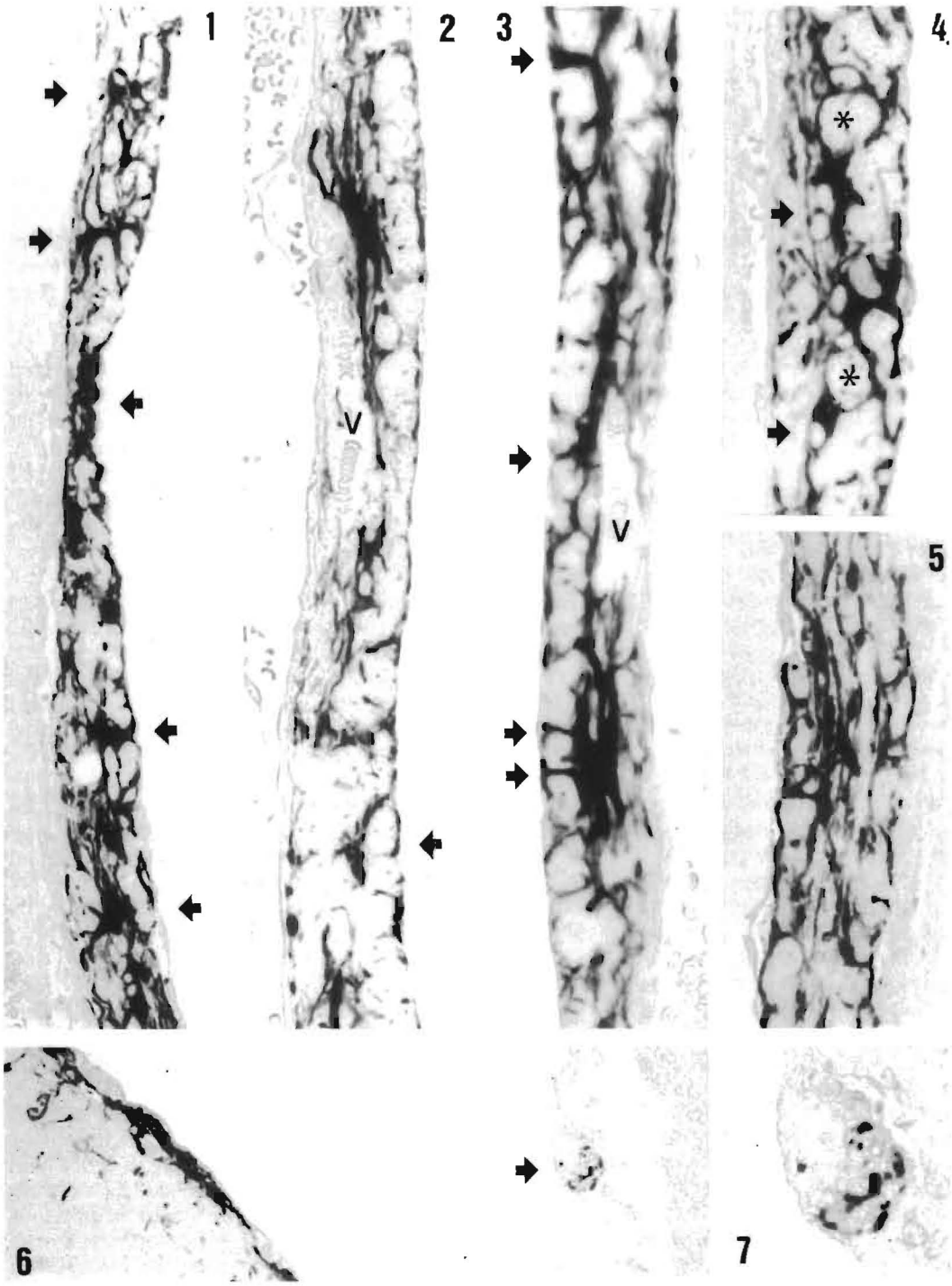

Fig. 1. Rat pineal stalk. Several immunoreactive cell somata are regularly spaced along the stalk length (arrows). x 315

Fig. 2. Rat pineal stalk. Stellate immunopositive cell showing many longitudinal processes. Some processes are located near a blood vessel (V) although a definite relationship cannot be observed. Arrows point to immunopositive processes encircling pinealocytes. x 510

Fig. 3. Rat pineal stalk. Immunopositive cell soma displaying longitudinal processes with small perpendicular branches. These branches frequently reach the stalk surface (arrow). V = blood vessel. x 550

Fig. 4. Rat pineal stalk. Several immunoreactive cell somata showing negative nuclei (arrows). Asterisks = pinealocytes encircled by immunopositive processes. x 450

Fig. 5. Rat pineal stalk. Numerous immunopositive processes forming a glial cover on the surface. x 480

Fig. 6. Cross-section of the pineal stalk (arrow) and adjacent brain tissue showing a strong positivity of the glia limitants. x 130

Fig. 7. Detail of Fig. 6. Cross-sectioned processes are strongly immunopositive. x 400

and showed an even distribution along the entire stalk. A tendency to a periodical regular arrangement of the immunoreactive cell somata, spacing at approximately each 125 μm, was observed (Fig. 1).

GFAP-immunopositive cells did not show a uniform morphology or size, although stellate-shaped cells showing variable number of cell processes were most noticeable (Figs. 2-4). An intense immunoreactive rim around the negative nucleus was commonly seen (Fig. 4). Immunoreactive cell processes showed diverse diameters and most of them were longitudinally oriented with respect to the stalk axis (Figs. 2, 3, 5) occasionally displaying sinuous profiles. In the transverse sections studied (Figs. 6, 7), cross-sectioned glial cell processes were clearly seen. However, the conspicuous existence of oblique or transverse processes with respect to the longitudinal stalk axis was also demonstrated in sagittal sections of the stalk, as was the presence of T-shaped branched glial cell processes (Figs. 3, 4). Over the surface of the stalk, glial cell processes were arranged in a glial cover layer that separated the pineal stalk from surrounding anatomical structures (Fig. 5).

GFAP-immunostained cells formed a wide framework within the entire length of the pineal stalk, supporting the remaining elements. Thus, pinealocytes were frequently encircled by GFAP-immunoreactive cell processes (Fig. 2), as were nerve fibres. Blood vessels were observed within the pineal stalk (Figs. 2, 3), although a definite relationship with glial cell processes was not evident.

Discussion

In the present work, the presence of astrocytes along the pineal stalk of the Wistar rat was demonstrated using an immunohistochemical technique for GFAP, a marker of mature glial cells in the central nervous system (Dahl and Bignami, 1986).

Two different cell types have been reported to be present in the rodent pineal gland parenchyma: pinealocytes, that are the main functional cell type; and a second cell type known as interstitial cell (Wolfe, 1965) or type II pinealocyte (Pevet, 1977). Several immunohistochemical studies using glial cell antigens such as GFAP, S-100 protein and vimentin have suggested a glial nature for this second pineal cell type (Moller et al., 1978; Schachner et al., 1984; Zang et al., 1985; Calvo et al., 1988a).

The ultrastructural features of the glial cells of the rat pineal gland are different to those of typical astrocytes of the central nervous system (Wolfe, 1965; Arstila, 1967; Pevet, 1977; Calvo and Boya, 1984). In this species, GFAP-immunoreactive cells are sparse and exclusively located near the pineal stalk (Calvo et al., 1988a).

GFAP-positive cells in the rat pineal stalk were strongly immunostained and display a stellate shape with a long and thick cell processed. Our results agree with previous ultrastructural observations by Luo et al. (1984a) and Calvo and Boya (1985) showing that this cell type is undistinguishable from mature astrocytes of the central nervous system. Luo et al. (1984a) noted that pineal stalk astrocytes show a higher cytoplasmic differentiation than glial cells within the pineal gland, with cell processes stuffed with filaments. According to our findings, the presence of clustered astrocytes as described by Luo et al. (1984a) was not confirmed. On the contrary, isolated astrocytes were regularly lined along the pineal stalk. This singular arrangement and the presence of a network of cell processes encircling the remaining elements would support a sustentacular role for this cell type.

Our results also confirm former ultrastructural descriptions on the discontinuous glial sheet limiting the entire pineal stalk (Luo et al., 1984a). This glial cover would be encircled by several leptomeningeal cell layers forming a continuous envelope that encloses all the pineal stalk. A narrow connective tissue space is interposed between the leptominengeal cell layers and the basal lamina that limits the pineal stalk tissue (Calvo and Boya, 1985).

On the other hand, the topographic location of the pineal gland and the type of innervation are factors that may influence the maturation of glial pineal cells. Thus, in mammals such as carnivores, with a deeply sited pineal organ (type AB of Vollrath, 1981), the ultrastructural characteristics of the second pineal cell type correspond to astrocytes (Hulsemann, 1967; Sheridan and Reiter, 1973; Calvo et al., 1988b). In these mammals, central nerve fibres are the main innervation (dog and cat: Tubahara, 1955) and are evenly distributed throughout the gland (Nielsen and Moller, 1975; Matsuura et al., 1983). Nevertheless, pineal glands in rodents are sited very superficially (type C of Vollrath, 1981) and the predominant innervation derives from sympathetic fibres arising in the superior cervical ganglia (Kappers, 1960; Bowers et al., 1984). In these latter species commisural fibres running through the pineal stalk, are restricted to the proximal area of the gland (Kappers, 1960; Luo et al., 1984b; Reuss and Moller, 1986; Mikkelsen and Moller, 1990). Interestingly, GFAP-immunopositive cells are exclusively located in both the proximal region and the pineal stalk. This finding may support that a maturative anteroposterior gradient could be present in the rat pineal organ and that the maturative stimulus would be carried by central nerve fibres reaching the pineal stalk and parenchyma. This hypothetical neuronal effect on the regulation of astroglial maturation has been suggested in several studies (Sturrock, 1986; Hatten and Mason, 1986). According to our results, mature astrocytes are exclusively found in those areas where central nerve fibres are located, i.e., the pineal stalk and the proximal region of the rat pineal gland.

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