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Presence of Glial Cells in the Rat Pineal Gland: A Light and Electron Microscopic Immunohistochemical Study

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ABSTRACT Immunoperoxidase methods for the demonstration of three glial antigens, vimentin, glial fibrillary acidic protein, and S-100 protein, were applied to routine-fixed paraffin sections of rat pineal gland. A pre-embedding electron microscope immunoperoxidase method was also used to study the ultrastructural localization of S-100 protein in pineal cells. Light and electron microscopic results showed the presence of these antigenic glial markers in the second pineal cell type. The term glial cell is proposed for the second of parenchymatous cell in rat pineal gland.

The mammalian pineal gland contains two types of parenchymal cells (Vollrath, 1981; Karasek, 1983). The first type of pinealocyte is the most abundant parenchymal cell, probably responsible for secretory functions in the pineal gland (Quay, 1974; Pevet, 1979). Several terms, such as interstitial or glial cells, have been used to designate the second pineal cell type (Wolfe, 1965; Vollrath, 1981; Karasek, 1983). In previous studies on the rat pineal gland, we have used the term type II pinealocytes for these cells (Calvo and Boya, 1983; 1984a,b, 1985; Boya and Calvo, 1984).

An astroglial nature has been proposed in some animal species for the second pineal cell type using immunofluorescent antibodies against glial markers such as vimentin, glial fibrillary acidic (GFA) protein, and S-100 protein (Møller et al., 1978; Huang et al., 1984; Schachner et al., 1984; Cozzi, 1986). Vimentin is a protein subunit of intermediate filaments initially described in mesenchymal cells and later found in glial cells, mostly astrocytes, ependymal, and Schwann cells (Schnitzer et al., 1981; Gown and Gabbiani, 1984). Vimentin appears early in the development of astrocytes, before the expression of GFA protein (Schnitzer et al., 1981). GFA protein is the main component of glial filaments. It is considered as an antigenic marker of astrocytes (Gown and Gabbiani, 1984), S-100 protein is a cytoplasmic calcium-binding protein described in several neuroectodermal cell types, including astrocytes (Nakajima et al., 1984).

Pineal cells positive for these glial antigens present star-shaped morphology and close proximity to connective tissue spaces. Consequently, they were identified as the second cell type of pineal parenchyma. Nevertheless, immunofluorescent techniques on unfixed cryostat sections yield poor morphological detail, making difficult the comparison with pineal morphology as shown by routine light microscope techniques. Moreover, the presence of glial antigen markers in the second pineal cell type has not yet been confirmed with electron microscopy.

The present investigation was undertaken to achieve a more definite demonstration of the glial nature of the second parenchymatous cell type in the rat pineal gland. Immunoperoxidase methods were applied to demonstrate vimentin, GFA, and S-100 protein on routine-fixed paraffin sections. A pre-embedding method for demonstration of S-100 protein at the electron microscope level was also used. S-100 protein, a cytoplasmic matrix protein, was chosen for immuno-electron microscope study assuming that it should provide a specific cytoplasmic staining of the second pineal cell type.

MATERIALS AND METHODS

Fifteen adult albino rats (4 months old) maintained under routine laboratory conditions (light:dark 14:10, food and water ad libitum) were used in this study. Rats were sacrified by decapitation under anesthesia, and the pineal gland was quickly removed and fixed by immersion in the appropriate fixative. For light microscope studies, a fixation in ice-cold 10% formalin was used. Two pineal glands were fixed in methacarn (60% methanol, 30% chloroform, 10% glacial acetic acid) as recommended for vimentin demonstration (Gown and Vogel, 1984). After 4 hours of fixation, samples were washed overnight in 0.1 M cacodylate buffer and embedded in paraffin. Seven-micron-thick serial sections were obtained, and groups of three consecutive sections were mounted on different slides for demonstrating the three antigens studied.

Peroxidase-antiperoxidase (PAP) methods for GFA and S-100 proteins as well as an indirect immunoperoxidase method for vimentin were performed according to Taylor (1986). Polyclonal rabbit anti-cow GFA protein, polyclonal rabbit anti-cow S-100 protein, and monoclonal mouse vimentin were used as primary antisera diluted 1/500, 1/320, and 1/10, respectively. All antibodies were obtained from Dako Laboratories, Denmark. A nuclear counterstaining with hematoxylin was applied to the sections after diaminobenzidine reaction.

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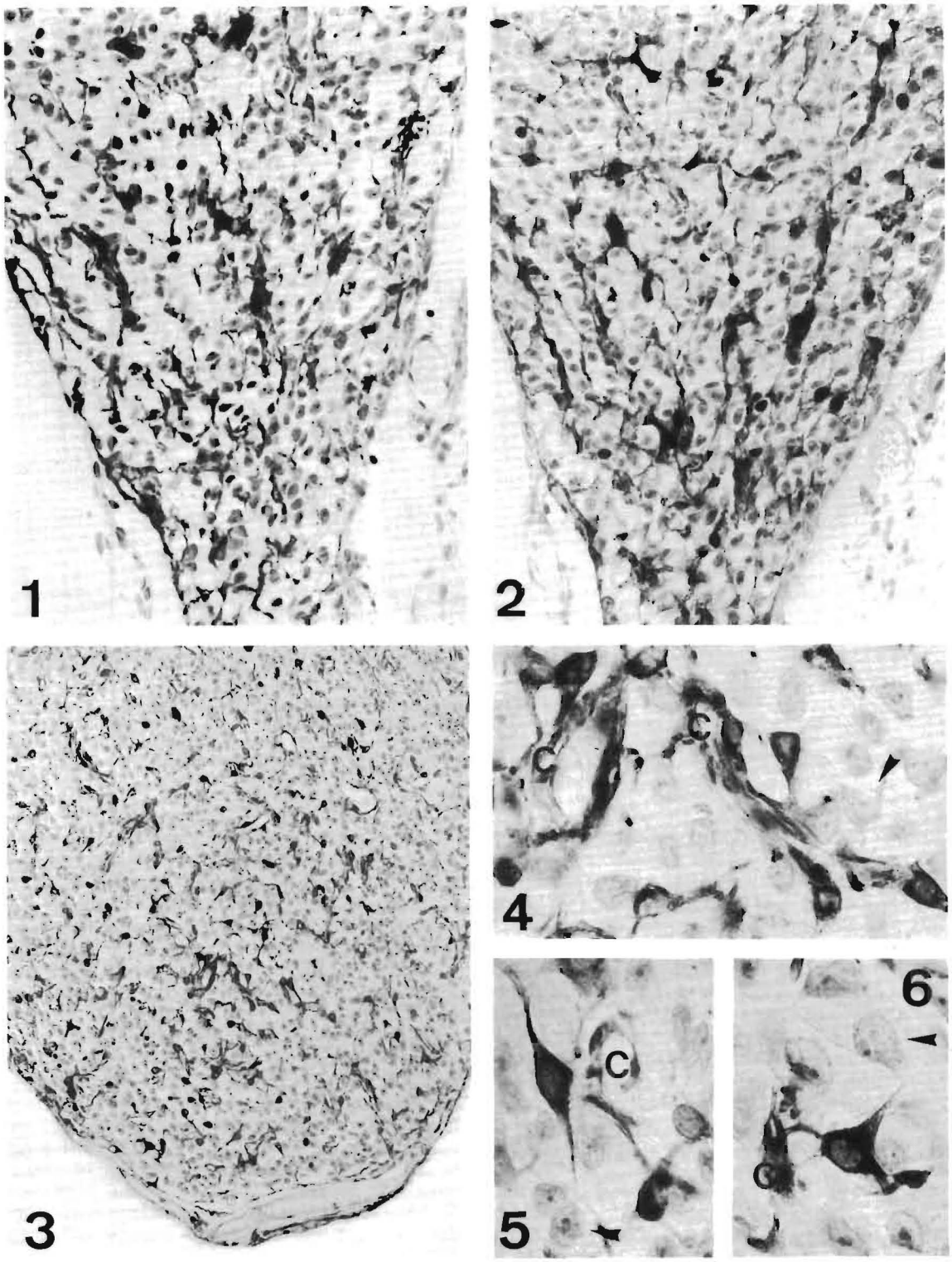
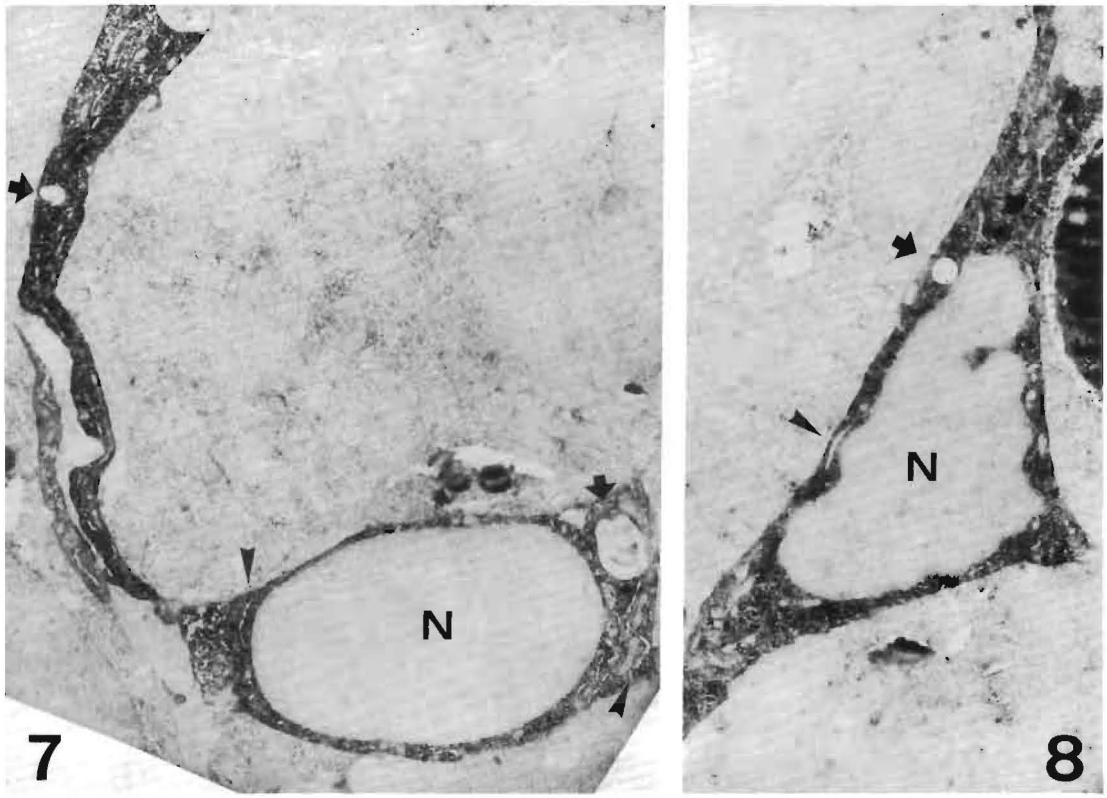


Fig. 1. Technique for GFA-protein demonstration. Proximal region of the pineal gland. Numerous star-shaped positive cells. ×360.

Fig. 2. Technique for S-100 protein demonstration. Proximal region of the pineal gland. Similar immunostaining pattern to that of GFA protein. ×360.

Fig. 3. Technique for vimentin demonstration. Distal region of the pineal gland. Positive cells are distributed throughout the gland. ×180.

Figs. 4–6. Technique for vimentin demonstration. Positive cells show small ovoid nuclei different from those of pinealocytes (arrowhead). Each positive cell sends out several processes directed toward connective tissue spaces (C). Some connective tissue elements, mostly blood vessels, are also stained. ×1,050.



Figs. 7, 8. Immunoelectron microscope demonstration of S-100 protein. Unstained ultrathin section. Pineal glial cells show nonstained nuclei and strongly positive cytoplasms. Rough endoplasmic reticulum cisternae (arrowheads) and some clear vacuoles (arrows) appear in glial cell cytoplasm. Osmiophylic lipid droplets can be seen in unstained pinealocytes. ×7,800.

For electron microscopy, pineal glands were fixed in ice-cold 0.5% glutaraldehyde-2% paraformaldehyde in 0.1 M phosphate buffer pH 7.4. After 4 hours of fixation, samples were washed overnight in 0.05 M Tris buffer-saline, pH 7.2. One hundred-micron-thick sections obtained in a Vibratome were incubated for S-100 protein demonstration following a pre-embedding method (Polak and Van Noorden, 1983). Once reacted with diaminobenzidine, sections were postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer and embedded in Vestopal. The first few ultrathin sections were selected from the surfaces of tissue blocks to overcome the low degree of penetration of the immunohistochemical methods. Unstained ultrathin sections were observed in a Philips EM 201 electron microscope.

RESULTS

Light Microscopy

The PAP methods for demonstration of both GFA and S-100 proteins showed an almost identical immunostaining pattern in the rat pineal gland (Figs. 1, 2). Numerous star-shaped cells appeared throughout the proximal half of the pineal gland. Both the number of stained cells and the cell-staining intensity were largely predominant in the pineal stalk as well as in the proximal half of the pineal gland (Figs. 1, 2). Conversely, with the immunohistochemical method for vimentin, positive cells were uniformly distributed throughout the gland (Fig. 3). A more intense immunostaining was found after methacarn fixation as compared with formalin-fixed tissues. Nevertheless, the same distribution of vimentin-positive cells was observed with both fixatives.

Positive cells for any of the three antigens detected in this study showed a similar morphology and localization. After comparing serial sections, the distribution of immunostained cells for these antigens was the same at least in the proximal half of the pineal gland. Immunostained cells presented small, dense ovoid nuclei easily distinguished from large vesicular nuclei showing conspicuous nucleoli characteristics of pinealocytes (Figs. 4–6). From each positive cell body two to five processes emerged, which were often directed toward connective tissue spaces (Figs. 4–6). Vimentin-positive cells could be seen in pineal connective tissue spaces, corresponding mostly to the endothelial cells of blood vessels (Figs. 4–6). Mesenchymal cells of pineal connective tissue spaces did not react with antibodies against either GFA or S-100 proteins.

Electron Microscopy

The pre-embedding method of Polak and Van Noorden (1983) for immuno-electron microscopy demonstrates the presence of S-100 protein in the second pineal cell type. Immunostained cells showed a strong cytoplasmic positivity, whereas their nuclei remained unstained (Figs. 6, 7). Differences in staining intensity were sometimes

found among adjacent cells. S-100 protein-positive cells showed an elongated or star-shaped cell body from which thin lamellar processes emerged (Figs. 7, 8). Often the cell body and/or its processes were in contact with connective tissue spaces. We could observe cisternae of rough endoplasmic reticulum and a special type of vacuole (Figs. 7, 8) previously described as characteristic of the second pineal cell type (Calvo and Boya, 1983, 1984b). Pinealocytes, identified by their conspicuous nucleoli and large cytoplasms, were always unstained.

DISCUSSION

According to our results, a population of cells in the rat pineal gland expresses three glial markers, vimentin, GFA and S-100 proteins. The amount of vimentin-positive cells throughout the pineal gland as well as that of GFA and S-100 proteins in the proximal half of the gland correlates with the percentage of second pineal cell type (10–15% of parenchymal cells) previously described (Calvo and Boya, 1984a).

The immunoperoxidase methods on routine-fixed paraffin sections used in this study give good morphological detail allowing an immediate correlation with previous light microscope findings. Positive cells for all of the three antigens studied showed the same morphological features and tissue distribution and relationships previously described for the second pineal cell type at the light microscope level (Calvo and Boya, 1984a).

An ultrastructural demonstration of the presence of S-100 protein in the second pineal cell type has been obtained for the first time in this study. The tissue localization, cell morphology, and cytoplasmic organelles present in S-100 protein-positive cells coincide with the ultrastructural features of the second pineal cell type we have previously described (Calvo and Boya, 1983, 1984b; Boya and Calvo, 1984).

Therefore, according to the present results, the second pineal cell type expresses vimentin, GFA and S-100 proteins. Main parenchymatous cells or pinealocytes were always negative for these antigens. In previous studies (Calvo and Boya, 1983, 1984a,b, 1985; Boya and Calvo, 1984), we have used the term type II pinealocytes for the second pineal cell type. Because of the present evidence of a glial nature for this cell type, the term pinealocyte should be exclusively used for the main parenchymatous cells. The broad term glial cell may be applied to the second pineal cell type in species such as the rat. The term astrocytes would be only used in species in which this second cell type is more similar to the nervous tissue astrocytes (Vollrath, 1981; Karasek, 1983).

Pineal glial cells seem to be a nonhomogeneous cell population at least with respect to the antigens expressed. Most if not all glial cells are vimentin positive, whereas GFA protein and S-100 protein-positive cells were restricted to the proximal half of pineal gland. Similar results have been reported by Schachner et al. (1984) studying the expression of vimentin, GFA protein, and C1 antigen in rat pineal gland. The presence of vimentin and C1 antigen in pineal glial cells is considered by these authors as a sign of immaturity. On the contrary, GFA protein positivity identifies a more mature glial population. Cells similar to astrocytes have been described with the electron microscope in species with a deeply located pineal gland (Hüselmann, 1967; Sheridan and Reiter, 1973). These cells are, however, scarce or absent in species whose pineal gland is located superficially. In the hamster, astrocytes have been only found with the electron microscope in the deep pineal (Sheridan and Reiter, 1970). In the rat, astrocyte-like cells rich in filaments have been reported mainly in the pineal stalk (Luo et al., 1984; Calvo and Boya, 1985). The presence of a topographic gradient in the glial cell differentiation can be proposed in rat pineal gland. Thus, mature glial cells (numerous filaments, positive for vimentin and, specially, GFA and S-100 proteins) are found in the most proximal region of the gland, whereas more immature cells (paucity or absence of filaments, only positive for vimentin) are located distally.

The glial nature of the second pineal cell type can have important functional implications. Thus, in the pineal gland, this cell type may have functions similar to those of the glial cells in the nervous tissues. Pineal glial cells, in addition to a supporting function, may also play a role in exchanges of substance between the pineal parenchyma and blood. A morphological support for this function may be the close relationship between the pineal glial cells and the connective tissue spaces of the gland (Wolfe, 1965; Arstila, 1967; Calvo and Boya, 1983, 1984b).

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