Coexpression of vimentin and glial fibrillary acidic protein in glial cells of the adult rat pineal gland

López-Muñoz F, Calvo JL, Boya J, Carbonell AL. Coexpression of vimentin and glial fibrillary acidic protein in glial cells of the adult rat pineal gland. J. Pineal Res. 1992:12:145–148.

Abstract: In the present work, coexpression of vimentin (VIM) and glial fibrillary acidic protein (GFAP) is demonstrated in the glial cells of the adult rat pineal gland. Serial consecutive Epon semithin sections (0.5 μm thick) were alternately immunostained for VIM and GFAP. GFAP positive cells and processes were found in the proximal region of the pineal gland, near the pineal stalk. Most of these cells were also immunostained for VIM in adjacent semithin sections. The significance of the coexpression VIM-GFAP and the restricted location of GFAP positive cells is discussed in relation with the maturation of pineal glial cells.

Francisco López-Muñoz, Jose Luis Calvo, Jesús Boya, and Angel L. Carbonell

Department of Histology, Faculty of Medicine, University Complutense, Madrid, Spain

Key words: vimentin—glial fibrillary acidic protein—glial cells—pineal gland—rat—coexpression

Address reprint requests to Prof. Jesús Boya Vegue, Department of Histology, Faculty of Medicine, University Complutense, 28040 Madrid, Spain

Received November 5, 1991; accepted February 11, 1992.

Introduction

Astrocytes contain two different types of intermediate filament (IF) proteins, i.e., vimentin (VIM) [Dahl et al., 1981; Schnitzer et al., 1981] and glial fibrillary acidic protein (GFAP) [Eng et al., 1971; Bignami et al., 1972]. Vimentin, initially considered as characteristic for mesenchymal cells, has been described in several cells of the central nervous system including radial glia, immature astrocytes and ependymal cells [Dahl et al., 1981; Schnitzer et al., 1981].

In the course of maturation, the expression of IF proteins in astrocytes shows a sequential change. Thus, VIM is initially expressed and later replaced by GFAP [Lagenaur et al., 1980; Schnitzer et al., 1981]. However, in some regions of the central nervous system such as the cerebellum, the retina, and the optic nerve, or in reactive astrocytes, the coexpression of both antigens has been demonstrated [Schnitzer et al., 1981; Shaw et al., 1981; Schiffer et al., 1986; Takamiya et al., 1988; Calvo et al., 1990, 1991].

The pineal gland in rodents contains two types of parenchymal cells [Vollrath, 1981], pinealocytes, and a second cell type named the interstitial cell [Wolfe, 1965; Arstila, 1967]. The recent immunohistochemical demonstration of GFAP and VIM in this pineal cell type supports its glial nature [Møller et al., 1978; Schachner et al., 1984; Calvo et al., 1988]. VIM immunoreactive cells are evenly distributed within the pineal parenchyma; however, GFAP expression is confined to those glial cells located in the proximal region of the gland [Calvo et al., 1988].

The aim of the present work was to demonstrate the coexpression of these IF proteins in this pineal cell type using an immunohistochemical method on semithin serial sections [Calvo et al., 1990].

Materials and methods

Twelve adult Wistar rats of both sexes were used in the present study. Animals were kept under routine laboratory conditions (light:dark 14:10) with free access to food and water. Rats were killed by decapitation under ether anesthesia, and the pineal gland was quickly removed and fixed by immersion in 2% glutaraldehyde, 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 at 4°C. After fixation, each gland was longitudinally divided into two halves and washed in phosphate buffer. Glands were embedded in Epon without previous osmification. Serial semithin sections (0.5 μm) were cut with a LKB ultramicrotome. Sections were etched for 20 min with sodium ethoxide, incubated in nonimmune swine serum and immunostained alternatively for VIM and GFAP. VIM was detected by an indirect immunoperoxidase method, using a monoclonal mouse anti-human VIM antiserum (1:50). A peroxidase-antiperoxidase (PAP) tech-

nique was applied to detect GFAP using a polyclonal rabbit anti-bovine GFAP (1:300). All antibodies were obtained from Dako Laboratories, Denmark.

The occurrence of VIM and GFAP in the same cell profiles was studied examining serial immunostained sections with a camera lucida.

Results

GFAP immunostained cells were found in the proximal third of the pineal gland near the pineal stalk (Fig. 1). GFAP immunoreactive cells were sparse, star-shaped, and were usually located near connective tissue spaces. Numerous strongly-GFAP immunostained cell processes, transversally or longitudinally cut, were observed in this same region as well.

VIM immunoreactive cells were uniformly distributed throughout the pineal gland (Fig. 2). These cells showed a similar shape to GFAP immunopositive cells described above. VIM immunoreactive cross-sectioned processes, appearing as minute positive dots, were also frequently seen. The strong immunostaining of the glia limitans of the adjacent cerebral tissue constituted an intrinsic positive control for both antisera.

The study of serial semithin sections, immunostained alternatively for each antisera, confirmed the coexpression of VIM in most of GFAP immunostained structures of the proximal region (Figs. 3,4). VIM immunopositive and GFAP immunonegative cell bodies and processes were commonly found in all cases (Figs. 5,6), but the opposite pattern was only occasionally noted.

Discussion

According to our findings, GFAP and VIM are coexpressed in the same pineal cell type of the adult Wistar rat. The method employed, using semithin serial sections (0.5 μm), allowed us to compare accurately the expression of both antigens in the same cell.

Several immunocytochemical studies have established the glial nature of the second pineal cell type. Thus, diverse glial cell markers, including GFAP, S-100 protein, or VIM, have been demonstrated in several mammal species: humans [Papasozomenos, 1983; Min et al., 1987], equines [Cozzi, 1986], bovines [Zang et al., 1985] and rodents [Møller et al., 1978; Schachner et al., 1984; Huang et al., 1984; Calvo et al., 1988].

In the adult rat, glial pineal cells show differences in their antigenic pattern depending on their location. Most of these cells express VIM and are uniformly dispersed within the gland [Calvo et al., 1988]. VIM is considered a marker for immature cerebral glia [Dahl and Bignami, 1986]. On the other hand, very sparse GFAP immunoreactive cells are located close to the pineal stalk. In contrast to VIM, GFAP is a characteristic IF protein for mature astroglial cells [Dahl and Bignami, 1986].

Developing astrocytes in the central nervous system initially express VIM and C1 antigen, while GFAP and M1 antigen are synthesized later in the course of differentiation [Schnitzer et al., 1981; Sommer and Schachner, 1981]. The modification of the antigenic profile occurs during the first 2 postnatal weeks in astrocytes of the rodent's central nervous system. As a result, adult astrocytes are GFAP immunopositive and VIM immunonegative [Schnitzer et al., 1981; Pixley and De Vellis, 1984; Voigt, 1989]. Considering the different expression pattern of IF proteins in adult pineal glial cells, we suggest that the rat pineal gland exhibits a combination of glial elements in diverse maturational phases. Since GFAP is only present in few cells, our findings support the hypothesis [Schachner et al., 1984] of the immature character of most pineal glial cells in adult rats.

The topographic location of the gland and the presence of central nerve fibers are factors that may influence the extent of maturation of pineal glial cells. The level of penetration of commissural fibers into the pineal gland varies depending on species. Thus, in those mammals with a deep-seated pineal gland (type A) [Vollrath, 1981], these nerve fibers are widely dispersed throughout the gland (dog) [Matsuura et al., 1983]; (primates and cat) [Nielsen and Møller, 1975]. However, pineal glands in rodents such as the rat [Kappers, 1960; Reuss and Møller, 1986; Mikkelsen and Møller, 1990], the guinea-pig [Schneider et al., 1981], or the Mongolian gerbil [Møller, 1985; Mikkelsen et al., 1991] are located very superficially (type C) [Vollrath, 1981] and central fibers entering the gland through the pineal stalk are restricted to the proximal area. According to Tubahara [1955], commissural nerve fibers form the main innervation of the cat and the dog pineal glands, whereas sympathetic nerve fibers constitute the predominant innervation of the rat pineal gland. The possibility arises that central nerve fibers may induce the maturation of pineal glial cells. This hypothesis may explain why GFAP immunopositive mature glial cells are exclusively located in those areas where commissural nerve fibers are distributed. As these hypothetical stimuli would be more powerful in the central nervous system, they could induce an earlier and full maturation of brain astrocytes.

The coexpression of VIM and GFAP has been

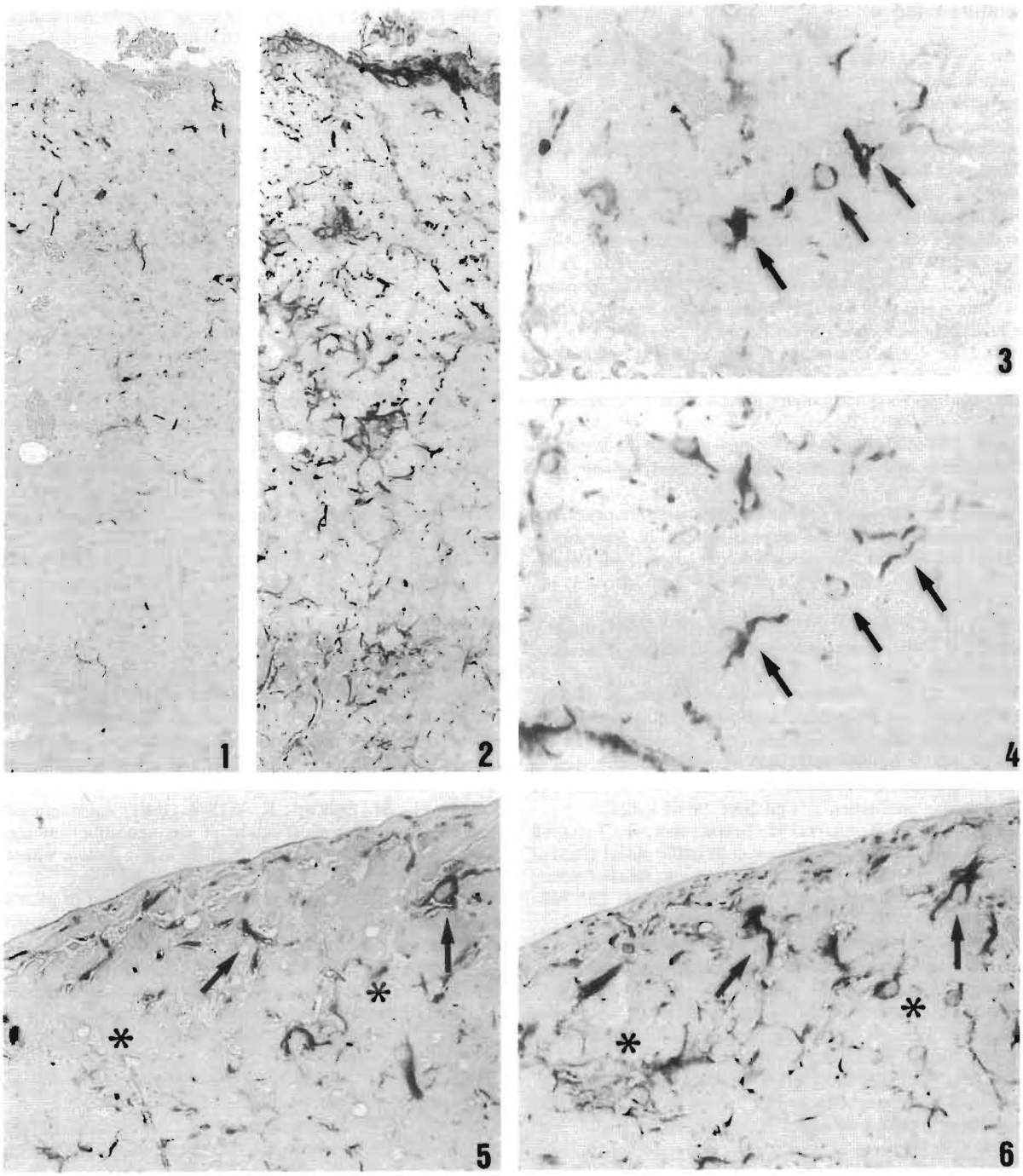

Fig. 1,2. Serial semithin sections of the proximal region of the pineal gland immunostained for GFAP (**Fig. 1**) and VIM (**Fig. 2**). ×250.

Fig. 3,4. Serial semithin sections showing the coexpression of GFAP (**Fig. 3**) and VIM (**Fig. 4**) in several glial pineal cells (arrows). ×620.

Fig. 5,6. Serial semithin sections immunostained for GFAP (**Fig. 5**) and VIM (**Fig. 6**). Some cell profiles coexpress both antigens (arrows). Scattered cell profiles are immunostained for VIM but not for GFAP (asterisks). ×500.

considered a sign of immaturity, and it has been repeatedly demonstrated that during maturation, astrocytes in the CNS express both IF proteins [Pixley and De Vellis, 1984; Voigt, 1989]. Assuming that commissural fibers elicit the transformation of pineal gland cells into astrocytes, such a stimulus presumably would be weak in the rodent pineal gland. This may account for the apparent relative immaturity of the astrocytes of the rat pineal gland even after adulthood.

Literature cited

ARSTILA, A.U. (1967) Electron microscopic studies on the structure and histochemistry of the pineal gland of the rat. Neuroendocrinology Suppl. 2:1–101.

BIGNAMI, A., L.F. ENG, D. DAHL, C.T. UYEDA (1972) Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. Brain Res 43:429–435.

CALVO, J.L., J. BOYA, A. BORREGON, J.E. GARCIÁ-MAURIÑO (1988) Presence of glial cells in the rat pineal gland: A light and electron microscopic immunohistochemical study. Anat. Rec. 220:424–428.

CALVO, J.L., A.L. CARBONELL, J. BOYA (1990) Coexpression of vimentin and glial fibrillary acidic protein in astrocytes of the adult rat optic nerve. Brain Res. 532:355–357.

CALVO, J.L., A.L. CARBONELL, J. BOYA (1991) Coexpression of glial fibrillary acidic protein and vimentin in reactive astrocytes following brain injury in rats. Brain Res. 566:333–336.

COZZI, B. (1986) Cell types in the pineal gland of the horse: An ultrastructural and immunocytochemical study. Anat. Rec. 216:165–174.

DAHL, D., A. BIGNAMI (1986) Intermediate filaments and differentiation in the central nervous system. In: Immunocytochemistry: Modern Methods and Applications. J.M. Polack, S. Van Noorden, eds. Wright and Sons Ltd., Bristol, pp. 401–412.

DAHL, D., C.J. CROSBY, A. BIGNAMI (1981) Filaments proteins in rat optic nerves undergoing wallerian degeneration. Exp. Neurol. 71:421–430.

ENG, L.F., J.T. VANDERHAEGHEN, A. BIGNAMI, B. GERSTL (1971) An acidic protein isolated from fibrous astrocytes. Brain Res. 28:351–354.

GOWN, A.M., A.M. VOGEL (1982) Monoclonal antibodies to intermediate filament proteins of human cells: Unique and cross reacting antibodies. J. Cell Biol. 95:414–424.

HUANG, S.-K., R. NOBILING, M. SCHACHNER, R. TAUGNER (1984) Interstitial and parenchymal cells in the pineal gland of the golden hamster. A combined thin-section, freeze-fracture and immunofluorescence study. Cell Tissue Res. 235:327–337.

KAPPERS, J.A. (1960) The development, topographical relations and innervation of the epiphysis cerebri in the albino rat. Z. Zellforsch. 52:163–215.

LAGENAUR, C., I. SOMMER, M. SCHACHNER (1980) Subclass of astroglia recognized in mouse cerebellum by monoclonal antibody. Devel. Biol. 79:367–378.

MATSUURA, T., M. KAWATA, H. YAMADA, M. KOJIMA, Y. SANO (1983) Immunohistochemical study on the peptidergic nerve fibers in the pineal organ of the dog. Arch. Histol. Jpn. 46:373–379.

MIKKELSEN, J.D., B. COZZI, M. MØLLER (1991) Efferent projections from the lateral geniculate nucleus to the pineal complex of the Mongolian gerbil (*Meriones unguiculatus*). Cell Tissue Res. 264:95–102.

MIKKELSEN, J.D., M. MØLLER (1990) A direct neural connection from the intergeniculate leaflet of the lateral geniculate nucleus to the deep pineal gland demonstrated with Phaseolus vulgaris leukoagglutinin (PHA-L) in the rat. Brain Res. 520:342–347.

MIN, K.W., I.S. SEO, J. SONG (1987) Postnatal evolution of the human pineal gland. An immunohistochemical study. Lab. Invest. 56:724–728.

MØLLER, M. (1985) Non-sympathetic synaptic innervation of the pinealocyte of the Mongolian gerbil (*Meriones unguiculatus*): An electron microscopic study. J. Neurocytol. 14:541–550.

MØLLER, M., A. INGILD, E. BOCK (1978) Immunohistochemical demonstration of S-100 protein and GFA protein in interstitial cells of rat pineal gland. Brain Res. 140:1–13.

NIELSEN, J.I., M. MØLLER (1975) Nervous connection between the brain and the pineal gland in the cat (*Felix catus*) and the monkey (*Cercopithecus aethiops*). Cell Tissue Res. 161:293–301.

PAPASOZOMENOS, S.C. (1983) Glial fibrillary acidic (GFA) protein-containing cells in the human pineal gland. J. Neuropathol. Exp. Neurol. 42:391–408.

PIXLEY, S.K., J. DE VELLIS (1984) Transition between radial glia and mature astrocytes studied with a monoclonal antibody to vimentin. Brain Res. 15:201–209.

REUSS, S., M. MØLLER (1986) Direct projections to the rat pineal gland via the stria medullaris thalami. An anterograde tracing study by use of horseradish peroxidase. Cell Tissue Res. 244:691–694.

SCHACHNER, M., S.-K. HUANG, P. ZIEGELMULLER, B. BIZZINI, R. TAUGNER (1984) Glial cells in the pineal gland of mice and rats. A combined immunofluorescence and electron-microscopic study. Cell Tissue Res. 237:245–252.

SCHIFFER, D., M.T. GIORDANA, A. MUGHELI, G. GIACCONE, S. PEZZOTTA, A. MAURO (1986) Glial fibrillary acidic protein and vimentin in the experimental glial reaction of the rat brain. Brain Res. 374:110–118.

SCHNEIDER, T., P. SEMM, L. VOLLRATH (1981) Ultrastructural observations on the central innervation of the guinea-pig pineal gland. Cell Tissue Res. 220:41–49.

SCHNITZER, J., W.W. FRANKE, M. SCHACHNER (1981) Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system. J. Cell Biol. 90:435–447.

SHAW, G., M. OSBORN, K. WEBER (1981) An immunofluorescence microscopical study of the neurofilament triplets proteins, vimentin and glial fibrillary acidic protein within the adult rat brain. Eur. J. Cell Biol. 26:68–82.

SOMMER, I., M. SCHACHNER (1981) Expression of glial antigens C1 and M1 in developing and adult neurologically mutant mice. J. Supramol. Struct. Cell Biochem. 16:53–74.

TAKAMIYA, Y., S. KOHSAKA, S. TOYA, M. OTANI, Y. TSUKADA (1988) Immunohistochemical studies on the proliferation of reactive astrocytes and the expression of cytoskeletal proteins following brain injury in rats. Brain Res. 38:201–210.

TUBAHARA, M. (1955) Über die Innervation der Epiphyse. Kurume Med. J. 2:218–234.

VOIGT, T. (1989) Development of glial cells in the cerebral wall of ferrets: Direct tracing of their transformation from radial glial into astrocytes. J. Comp. Neurol. 289:74–88.

VOLLRATH, L. (1981) The pineal organ. In: Handbuch der Mikroskopischen Anatomie des Menschen. A. Oksche, L. Vollrath, eds. Springer Verlag, Berlin, Vol VI/7.

WOLFE, D.E. (1965) The epiphyseal cell: An electron-microscopic study of its intercellular relationships and intracellular morphology in the pineal body of the albino rat. Prog. Brain Res. 10:332–386.

ZANG, X., G. NILAVER, B.M. STEIN, M.R. FETELL, P.E. DUFFY (1985) Immunocytochemistry of pineal astrocytes: Species differences and functional implications. J. Neuropathol. Exp. Neurol. 44:486–495.