The Presence of Glial Fibrillary Acidic Protein in the Human Pituitary Gland

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Abstract. The presence of glial fibrillary acidic protein (GFAP) was studied in human pituitary glands with the peroxidase-antiperoxidase (PAP) method. Positive reaction was observed in cells and processes of the neurohypophysis, in occasional cells lining the Rathke's cysts of the pars intermedia, and in scattered star-shaped cells and small follicles of the pars distalis. GFAP immunoreactivity was sparse and variable in amount from case to case. An increase in GFAP-immunoreactivity was observed as a reaction to injury. GFAP-positive cells were seen within and around pituitary adenomas regardless of their secretory cell type. Evidence is presented to indicate that these cells do not contain conventional pituitary hormones. It is postulated that the GFAP-positive cells of the pars distalis are nonsecretory elements, identical to the folliculostellate cells. They may become visible by immunostaining following increased synthesis of GFAP. The latter may be a response to cell injury or metabolic changes in adjacent secretory elements. A similar reaction in pituicytes may explain the appearance of immunoreactive GFAP in the neural lobe. The presence of GFAP in the adenohypophysis suggests that some of their cells are neuroectodermal in origin.

Key Words: Folliculostellate cells, Glial fibrillary acidic protein, Pituicytes, Pituitary adenomas, Pituitary gland, Rathke's cyst.

INTRODUCTION

The glial fibrillary acidic protein (GFAP), a structural protein of the nervous system, has been localized in normal and reactive astrocytes, immature and reactive ependymal cells, and in neoplastic cells of astrocytic and ependymal derivation (1, 2). The neurohypophysis, a direct anatomical extension of the basal hypothalamus, contains cellular glial elements, the pituicytes, which very much resemble cerebral astrocytes (3, 4). Descriptions of the topographical distribution of GFAP in the normal brain have been reported (2), but information about its presence in the posterior lobe of the hypophysis is lacking. To find out whether GFAP occurs in the neural component of the pituitary gland, we studied normal and pathological pituitary tissues following staining for GFAP with the peroxidase-antiperoxidase (PAP) method of Sternberger (5). GFAP was not only found in the neural lobe as expected, but was also detected in the glandular component of the pituitary. Preliminary results of this study

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were presented in abstract form (6). A full description of these findings is now the subject of this report.

MATERIALS AND METHODS

Pituitary tissue obtained at autopsy and after surgery was fixed in either formalin or Bouin’s fluid. A total of 36 non-neoplastic glands obtained at autopsy from adult individuals (18 years of age and older) were studied. Two sections or more of each gland were examined, one of them always involving the neural and glandular lobe in either horizontal or sagittal sections. The average postmortem interval was 8 hours, with a range from 1 to 48 hours. Three additional glands harboring incidental pituitary microadenomas were also studied. Surgical specimens comprised 10 non-neoplastic glands obtained following hypophysectomy for palliative therapy of advanced breast carcinoma, one pituitary obtained following resection of a hypothalamic germinoma with intrasellar extension, and 41 pituitary adenomas resected by transphenoidal microsurgery. Following paraffin embedding and tissue sectioning, the PAP method using rabbit anti-human GFAP serum was applied, essentially as described previously (7). The study was carried out with anti-human GFAP serum produced in this laboratory according to the method of Dahl and Bignami (2, 8). A few cases were also stained with antiserum raised against bovine GFAP, generously provided by Dr. L. F. Eng. Staining specificity was assessed by replacing the primary antiserum with normal rabbit serum or by deleting the primary antiserum from the staining sequence. The specificity of the primary antiserum was, in turn, evaluated by absorption with an excess of purified GFAP. A number of non-neural tissues were also stained for control purposes. They included: liver, pancreas, gastrointestinal tract, spleen, ovary, thyroid, kidney, adrenal gland, parathyroid, lung, heart, skin, and several mesodermal tissues (skeletal muscle, cartilage, bone, and adipose tissue). For endocrine immunocytochemical studies, the following sera were used: antisera to human growth hormone and human prolactin, kindly supplied by Dr. A. F. Parlow; and antisera to porcine ACTH and the β subunits of human FSH, human LH, and human TSH, obtained from the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD). The sections were stained with the PAP method as above, using similar controls for staining specificity. Absorption with their respective antigens was employed for evaluation of the specificity of the primary antiserum. Anti-GFAP serum was also absorbed with the same hormonal antigens. Identification of two antigens (GFAP and prolactin) in the same tissue section was accomplished, using the procedure described by Erlandsen et al. (9). Reaction products of two different colors were obtained by using 3,3′-diaminobenzidine (DAB) (brown) and 4-Cl-1-naphthol (CIN) (blue) to develop the peroxidatic reaction. After localization of the first antigen with DAB, the tissue-bound antibodies were eluted with 1 N HCl. The second antigen was then localized with CIN.

RESULTS

Positive GFAP immunoreaction was observed in the neurohypophysis and in cells of the pars distalis and the intermediate zone of the adenohypophysis. Identical topographical localization was observed with both GFAP antisera. Surgical and autopsy specimens showed a similar pattern of GFAP distribution (Table 1). All non-neural tissues examined were negative. Absorption with purified GFAP abolished the reaction. In contrast, no decrease in staining intensity was noted following absorption of anti-GFAP with pituitary hormones.
### TABLE 1
Occurrence of GFAP Immunoreactivity in the Normal Human Pituitary Gland

<table>
<thead>
<tr>
<th></th>
<th>Autopsy specimens</th>
<th>Surgical specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.*</td>
<td>%</td>
</tr>
<tr>
<td>Posterior lobe</td>
<td>22/36</td>
<td>61.1</td>
</tr>
<tr>
<td>Intermediate zone</td>
<td>25/36</td>
<td>69.4</td>
</tr>
<tr>
<td>Anterior lobe</td>
<td>21/36</td>
<td>58.3</td>
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</tbody>
</table>

* The presence of positive cells was studied in two or more sections per gland. Cases showing rare GFAP-immunoreactivity were considered negative.
† Due to the distorted anatomy of the gland, the neurohypophysis was identified in 9 of 10 cases, while pars intermedia Rathke's cysts were found in only 8 of 10 cases. Only one section of each fragment was examined. Multiple step sections were not attempted.

In the neurohypophysis, GFAP-positive immunoreaction most often occurred in fibrous processes (Fig. 1). Occasionally, isolated pituicytes also showed intense perinuclear immunoreaction. The stained elements were variable in number from case to case and haphazardly distributed. In several cases the GFAP immunoreaction was rare or altogether absent (Table 1). Loose tangles of tortuous GFAP-positive fibers were randomly detected. Occasionally, condensations of GFAP-positive fibers were seen around blood vessels, forming loose, irregular cuffs of coarse fibers (Fig. 1). Dense bundles of coarse GFAP-positive fibers admixed with cells showing intense perinuclear staining could also be seen in a few cases (Fig. 2). These structures did not appear to be arranged around blood vessels, and suggested areas of collapse of the native architecture in a pattern similar to the glial scars of the central nervous system.

The colloid-filled cysts (Rathke's cysts) of the intermediate zone were the sites in which GFAP-positive material was most consistently found (Table 1). The epithelium lining these cavities showed strongly-stained cells alternating with unstained elements (Fig. 3). Although not all the cysts contained GFAP-positive cells, most of them had strongly-stained elements not morphologically different from the unstained adjacent cells. Pituitary cell acini containing numerous GFAP-positive cells were occasionally found connected to the epithelium of the cysts (Fig. 4).

Isolated cells of the pars distalis showed strong cytoplasmic immunoreactivity (Fig. 5). These adenohypophysial-GFAP-positive cells (AGP cells) did not have a consistent distribution, and varied considerably in number from case to case (Table 1). Some specimens were totally devoid of AGP cells. The stained cells had a peculiar morphology, and their cellular profiles were unlike any other endocrine pituitary cell. The scanty perinuclear immunoreactivity could be followed into thin branching processes terminating in capillary sinuoids. Isolated fibrous processes without an apparent point of connection with a cellular soma were also seen frequently (Fig. 5). Sometimes, AGP cells
Fig. 1. GFAP-positive terminals of coarse fibrous processes surrounding a small blood vessel (V) of the neurohypophysis. GFAP-PAP. ×250.

Fig. 2. A patch of GFAP-immunoreactivity in the neurohypophysis. Coarse, irregular, wavy fibers and a few pituicytes showing cytoplasmic immunoreaction around the unstained nuclei (arrows) are present. Bulbous dilatations of some fibers mimic the pattern observed in Rosenthal fibers (7) (arrowhead). GFAP-PAP. ×250.
Fig. 3. Alternating pattern of GFAP staining in the lining epithelium of a Rathke's cyst. NH, neurohypophysis; AH, adenohypophysis. GFAP-PAP. ×250.

were part of the wall of small colloid cysts with processes projecting toward the lumina.

Some pathological conditions of the adenohypophysis were associated with morphological changes in AGP cells. Considerable hyperplasia and hypertrophy of these cells were observed in the vicinity of a recent microinfarct in an autopsy specimen (Fig. 6). Marked hypertrophy and hyperplasia of AGP cells were also seen in the nontumorous adenohypophysis obtained at surgery, three months following removal of a pituitary adenoma (Fig. 7). Organized scars of the adenohypophysis, in contrast, were not associated with changes in these cells. AGP cells were especially prominent in the glandular parenchyma bordering pituitary adenomas. A rim of intensely-stained cells formed an almost continuous band at the interface between the neoplasm and the adjacent normal gland (Fig. 8). This peculiar arrangement of strongly-stained cells was well demonstrated in ten pituitary adenomas (one growth hormone cell adenoma, three prolactin cell adenomas, and six pituitary adenomas with negative immunoreaction for all hormones), and in the adenohypophysis bordering the front of invasion of a hypothalamic germinoma. The edge of the adenoma could not be clearly identified in most of the remaining cases, due to either distortion of the architecture or absence of adjacent normal gland within the specimen. All three adenomas obtained at autopsy exhibited prominent AGP cells in the periadenomatous zone. Some adenomas contained GFAP-positive cells scattered at random within the tumor mass (Fig. 9). Adenomas containing colloid-filled follicular formations showed intense staining of isolated cells lining the wall of the cavity (Fig. 10). These follicles were more frequent near the periphery of the tumor, and their pattern of staining closely resembled that observed in the Rathke's cysts of the pars intermedia and that of occasional follicular formations within the nontumorous pars distalis. AGP cells were observed in 23 of 41 adenomas obtained at surgery, and the occur-
Fig. 4. Pars intermedia zone containing isolated GFAP-positive cells in the Rathke's cyst (C) epithelium and in adjacent, solid clusters of cells (arrowheads). Some of the latter are connected to the cyst epithelium (arrows). NH, neurohypophysis. GFAP-PAP. ×250.

rence of positive cells was not peculiar to any type of endocrine cell neoplasm (Table 2). One case was particularly interesting because of the profusion of AGP cells within the tumor. Hormonal immunocytochemical studies revealed that the adenoma was formed by a uniform population of prolactin (PRL) cells, with only rare growth hormone (GH) and ACTH cells and no TSH, LH, or FSH elements. Double staining of the same tissue section using two different capturing agents in succession indicated that GFAP and PRL occurred in two different cell populations, a notion initially hinted at by the different outline of the cells obtained following staining with each antiserum. The lack of hor-
Fig. 5. Star-shaped cells and isolated fibrous processes (arrow) are a common pattern of GFAP staining in the pars distalis. GFAP-PAP. ×250.

monal antigens in AGP cells was also indicated by the failure to localize GH, PRL, ACTH, LH, FSH, or TSH in five adenomas containing strongly-stained AGP cells. Two other undifferentiated adenomas showing AGP cells contained few scattered LH and FSH cells, which could be easily differentiated from the former by their different morphology and location.

DISCUSSION

This study demonstrates that GFA protein, a well-recognized structural protein of the central nervous system, can also be found in the human pituitary gland. Its presence in the neurohypophysis was expected, considering the central nervous system derivation of this portion of the gland. Only a few cells showed positive reactions, however, and they were haphazardly distributed throughout the gland. The lack of an exhaustive search for GFAP-containing pituicytes in serial sections may explain the negative findings in many cases. The irregular GFAP distribution was not secondary to preservation of the antigen, since the pattern was the same regardless of the postmortem interval and not different in surgical specimens where the fixation was prompt and regular. It is likely that it may reflect variable metabolic states of the pituicytes triggered by either focal cell injury and degeneration or variations in the degree of secretory activity within the neural lobe. Cellular activation similar to the
central nervous system glial response has been seen in the pars nervosa of experimental animals following surgical interruption of the hypothalamic-hypophyseal tract (10). Activation of pituicytes involving increased mitotic activity, increased protein synthesis, and cellular hypertrophy have also been demonstrated following stimuli known to induce neurohypophysial secretion (11). It can be speculated that this cellular activation also involves increased synthesis and accumulation of GFAP. The absence of demonstrable GFAP in most of the pituicytes does not in any way negate their postulated parentage with central nervous system astroglia. This feature, in fact, is reminiscent of the GFAP-negative reaction of most astroglial cells in the gray matter (protoplasmic astrocytes). Interestingly enough, the latter respond to injury with increased synthesis of GFAP, as revealed by their transformation into GFAP-positive cells following a stab wound (12). A potentiality for increased synthesis and accumulation of GFAP may be a property of pituicytes as well.

The presence of GFAP-positive cells in the adenohypophysis was unexpected. Here, again, the incidence of GFAP-positive cells varied from gland to gland. Their morphology and spatial arrangement among endocrine cells, capillaries, and pools of colloid, however, was consistent. They resemble, in this respect, the follicular and stellate cells of Farquhar (13). These two cell types are identical in humans and, therefore, are commonly known as folliculostellate (FS) cells. Like the FS cells, the adenohypophysial-GFA-positive cells (AGP cells) do not appear to contain pituitary hormones. We found AGP
cells in all types of pituitary adenomas, including those without hormonal immunoreactivity [undifferentiated or "null" cell adenomas (14)]. Further proof of the nonendocrine nature of these cells was provided by the successive staining of GFAP and PRL in the same tissue section of a prolactin cell adenoma. This experiment demonstrated the presence of GFAP in a large population of cells totally devoid of PRL immunoreactivity. Since the tumor was essentially negative for all other pituitary hormones, it can be concluded that the PRL-negative-GFAP-positive cells lacked these other pituitary hormones as well. In view of these facts, we believe that AGP cells are truly nonendocrine in nature. No secretory cell of the adenohypophysis shows the cellular profile of the AGP cells and, conversely, no other pituitary cell reproduces so well the morphological features attributed to FS cells. Since the FS cells possess characteristic ultrastructural features (lack of granules, desmosomes, intermediate filaments, etc.), the final proof of identity must await the demonstration of GFAP in typical FS cells characterized by electron microscopy.

Although the functional significance of the FS cells remains unresolved, most authors suggest that they serve a sustentacular function, i.e., they form a supportive framework which keeps secretory cells and capillaries in place—a
Fig. 8. Prominently-stained AGP cells bordering a prolactin-cell pituitary adenoma (PA). GFAP-PAP. ×30.

Fig. 9. GFAP-positive cells within the tumor mass of an undifferentiated pituitary adenoma obtained at surgery. GFAP-PAP. ×250.
Fig. 10. Colloid-filled follicular formation in an undifferentiated pituitary adenoma. Many isolated GFAP-positive cells are seen within the walls of the cavity. GFAP-PAP. \( \times 250 \).

function, therefore, not unlike that of astrocytes in the central nervous system (13). Their spatial arrangement between capillaries (to which they project end-foot processes) and the secretory cells suggests a transport role as well, a hypothesis supported by the incorporation of horseradish peroxidase into FS cells when injected systemically (15). Furthermore, the localization of ATPase in their cell membranes had led some investigators to believe that FS cells may serve as regulators of the extracellular ionic environment of the secretory cells (16). A similar role was suggested earlier for astrocytes with respect to neurons. The morphological and functional similarity of FS cells and astroglial cells has led several investigators to refer to them as glial-like (16, 17) or glial cells (18) of the pituitary. The presence of GFAP in FS cells may provide further support to the concept that these cells are closely related and perhaps identical to astroglial cells. This view has been recently reinforced with the demonstration of S-100 protein, a central nervous system protein of mainly glial origin, in the rat FS cells (17, 19).

It is likely that FS cells have an important function in the pituitary secretory process. Changes in size and number of FS cell processes were described in a number of states in which pituitary endocrine cells show enhanced secretory activity (20, 21). In addition to the supply of materials to the endocrine cells, the digestion of waste material from actively secreting cells may lead to morphological changes. It is possible that functional activation of these cells may
also be associated with an enhanced accumulation of GFAP. The variable occurrence of GFAP immunoreaction from case to case may therefore reflect a different physiological state of the secretory process in the gland.

Enhanced synthesis and accumulation of GFAP may also occur as a reaction to injury, as suggested by the pattern of staining observed around recent pituitary infarcts, mechanical disruption following surgery, or compression due to the growth of an expanding mass within the adenohypophysis. The changes thus resemble the glial reaction following central nervous system damage. It has been suggested that the colloid-filled follicles occasionally found within the human pars distalis may be transitory structures representing enclosed foci of cell degeneration and necrosis (22). The walls of these follicular structures consist of a variety of endocrine cell types, and intercalated among them there are cells ultrastructurally identical to the FS cells of Farquhar. They contain cytoplasmic intermediate filaments which may occasionally be very abundant. We think that these are the GFAP-containing cells identified in our preparations and that they contain, therefore, intermediate filaments immunohistochemically identical to those of fibrous astrocytes. The AGP cells lining the Rathke’s cysts could also be reactive follicular cells. Their enhanced GFAP production could be triggered by products of cell degeneration present within these cavities. The ultrastructural homology of “marginal cells” along the hypophysial clefts with ordinary follicular cells found elsewhere within the pars distalis has been recognized in the mouse adenohypophysis (20). These cells undergo similar morphologic alterations following changes in the physiological state of the animal. It was suggested that these changes are related to the appearance of colloid in follicular cavities and in the hypophysial cleft. The colloid most likely consists of degradation and waste material from activated endocrine cells. In summary, we believe that the stellate cells, follicular cells, and marginal cells of the adenohypophysis are different forms of the same cell type and that they

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**TABLE 2**
Occurrence of AGP Cells in Human Pituitary Adenomas

<table>
<thead>
<tr>
<th>Predominant cell type*</th>
<th>No.†</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>1/2</td>
<td>50.0</td>
</tr>
<tr>
<td>GH</td>
<td>1/4</td>
<td>25.0</td>
</tr>
<tr>
<td>PRL</td>
<td>14/19</td>
<td>73.6</td>
</tr>
<tr>
<td>Mixed PRL/GH</td>
<td>0/2</td>
<td>0</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>7/14</td>
<td>50.0</td>
</tr>
<tr>
<td>Total</td>
<td>23/41</td>
<td>56.0</td>
</tr>
</tbody>
</table>

* Immunocytochemical localization of ACTH, GH, PRL, βLH, βFSH, and βTSH was studied in one prolactin cell adenoma and in fourteen undifferentiated adenomas. In the remaining twenty-six tumors, ACTH, GH, and PRL were the only hormones studied.
† Cases with only rare AGP cells were not counted as positive.
respond in the same manner, increasing the synthesis and accumulation of GFAP, in response to events taking place in the adjacent endocrine cells. The alternating pattern of GFAP staining of the Rathke's cysts lining epithelium, so similar to that of primitive (23), reactive (24), or neoplastic ependyma (7, 24, 25), may suggest an embryologic relation with the third ventricle ependyma. In fact, a neuroepithelial derivation for some of the cells lining the spaces in the intermediate zone of the pituitary gland has previously been proposed (26). Although our findings are compatible with the view that pinched-off remnants of reactive ependymal cells lie within the gland, we favor the hypothesis that these GFAP-containing cells are just another form of reactive follicular cells.

The occurrence of a nervous tissue-specific protein in the adenohypophysis is not unprecedented. Neuronal-specific enolase (27) and S-100 protein (17, 19) have previously been demonstrated by immunocytochemistry in the rodent pituitary. The presence in this portion of the pituitary of elements containing proteins considered unique to the nervous system provides additional support to the view of Takor and Pearse (28) that not only the neural lobe but also the adenohypophysis derives from the neuroectoderm. If it can be unmistakably shown that these proteins are synthesized in situ by native hypophysial cells, a neuroectodermal derivation for at least some adenohypophysial cells will have to be assumed.

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Note added in proof: Following submission of the present manuscript, a paper reporting that rat pituicytes contain GFAP was published by Suess U and Pliska V (Brain Res 1981;221:27-33).

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