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Localization of fibronectin in the folliculo-stellate cells of the rat anterior pituitary by the double bridge peroxidase-antiperoxidase method

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Summary. The localization of fibronectin was demonstrated in the rat anterior pituitary by the highly sensitive double bridge peroxidase-antiperoxidase (PAP) method. The fibronectin immunopositive cells were characterized by stellate-like morphology. The cells immunostained for fibronectin were observed to be identical to those for S-100 protein in adjacent mirror sections, whereas the S-100 protein has been specifically immunodetected in the folliculo-stellate (FS) cells of the anterior pituitary. The present study indicates that the fibronectin is present in the FS cells, suggesting that FS cells might play a role in the regulation of pituitary function through the interaction of fibronectin with hormone secreting cells

Introduction

Folliculo-stellate (FS) cells of the anterior pituitary are a population of nongranular cells with thin cytoplasmic projections extending between granulated cells and with follicle-like structures formed among themselves (Rinehart and Farquhar 1955; Vila-Porcile 1972). Numerous investigations have suggested that FS cells could be involved in various cellular functions, such as supportive function (Salazar 1968), stem cell function (Yoshimura et al. 1977), ion transport (Ferrara et al. 1987a), phagocytosis (Stokreef et al. 1986), and paracrine regulation of hormone secreting cells (Baes et al. 1987). S-100 protein, originally isolated from the brain, was found to be present in the FS cells (Nakajima et al. 1980). Recently, cultured FS cells were shown to synthesize fibroblast growth factor (FGF) (Ferrara et al. 1987b) and the results provided additional evidence on the multifunctional roles of FS cells in the regulation of the anterior pituitary.

In the present communication, we report the localization of fibronectin in the FS cells of the rat anterior pituitary and the coexistence of fibronectin with S-100.

Materials and methods

Tissue preparation. Six-week-old female Fischer rats were used in the present study. After decapitation under the anesthesia with ether, the pituitaries were fixed in 4% paraformaldehyde-1% picric acid in phosphate buffer (0.1 *M*, pH 7.4) overnight at 4° C, dehydrated in a series of graded ethanol and embedded in paraplast. Serial frontal sections 2 μm thick were made, mounted onto gelatin-coated slides, deparaffinized, and hydrated through a series of graded ethanol.

Immunohistochemistry. The double bridge peroxidase-antiperoxidase (PAP) technique of Vacca et al. (1980) was employed for the immunostaining with a slight modification. Briefly, after pretreatment with 0.5% H_2O_2 in methanol for 30 min in order to eliminate the effect of endogenous peroxidase, the sections were incubated with antisera at room temperature in the following sequences: *1)* either rabbit anti-fibronectin serum (Advance, Tokyo) diluted 1:500 or rabbit anti-S-100 serum (IBL, Gunma) diluted 1:500; *2)* goat anti-rabbit IgG serum (GAR) (provided by Dr. Wakabayashi, Hormone Assay Center in our institute) diluted 1:5,000; *3)* rabbit PAP complex (Dako) diluted 1:200; *4)* the second application of GAR diluted 1:10,000; *5)* the second application of rabbit PAP complex diluted 1:200. Finally, the sections were incubated with 0.05% diaminobenzidine tetrahydrochloride (DAB)-0.03% H_2O_2 in *Tris* buffer (0.05 *M*, pH 7.4) for 10 to 20 min.

The specificity of the immunostaining was tested by (*a*) applying the first antibody preabsorbed with either homologous or heterogenous antigen (fibronectin, Sigma; S-100 protein, Wako; 10 μg/ml of 1:500 diluted antibody) and (*b*) omitting either the first antibody or the second antibody.

Results

Fibronectin-immunoreactive cells were specifically detected in the rat anterior pituitary (Fig. 1). The immunoreactive cells were irregular, stellate-like in shape, present individually or in clusters. Marginal layer cells of the pars intermedia were also immunoreactive. The stained stellate-shaped cells possesed thin cytoplasmic processes extending between the secretory cells.

In the adjacent sections, a very similar distribution was observed between fibronectin-positive cells and S-100 protein-positive cells (Fig. 2). Cells immunostained for fibronectin were demonstrated to be identical to those for S-100 protein in the mirror sections (Fig. 3).

Neither fibronectin antiserum nor S-100 protein antiserum preabsorbed with homologous antigen showed immunospecific staining, whereas the immunoreaction was

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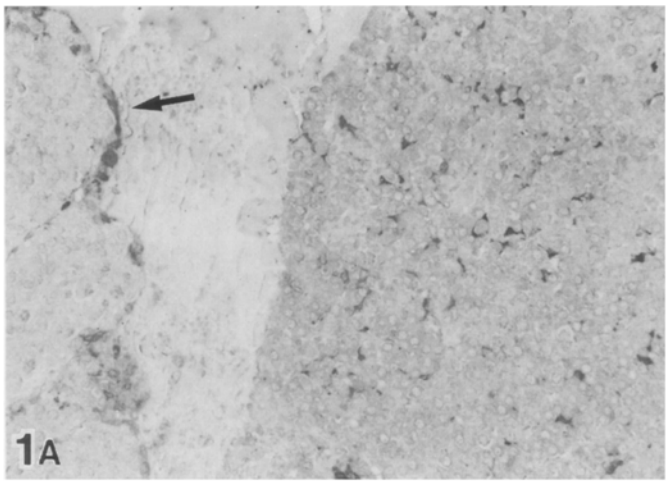

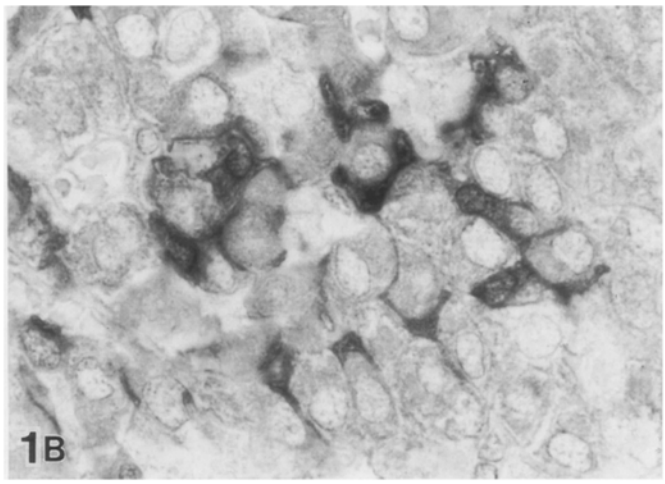

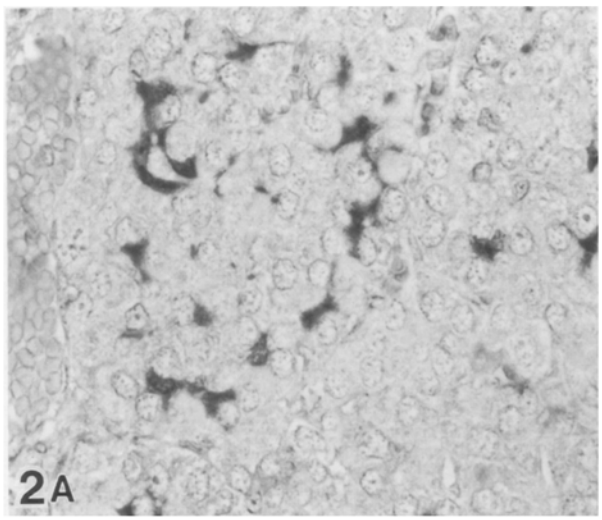

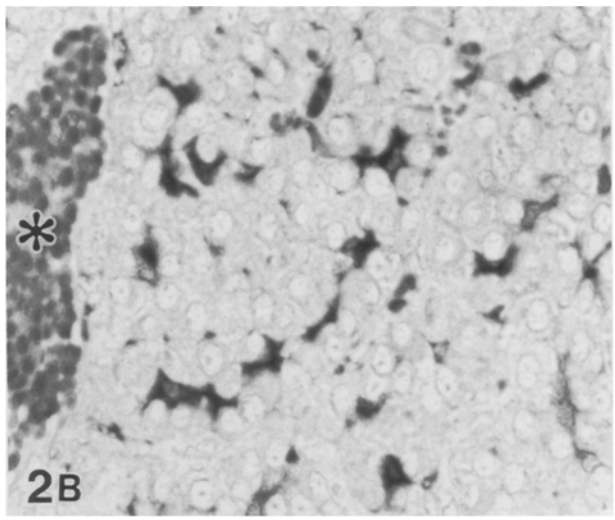

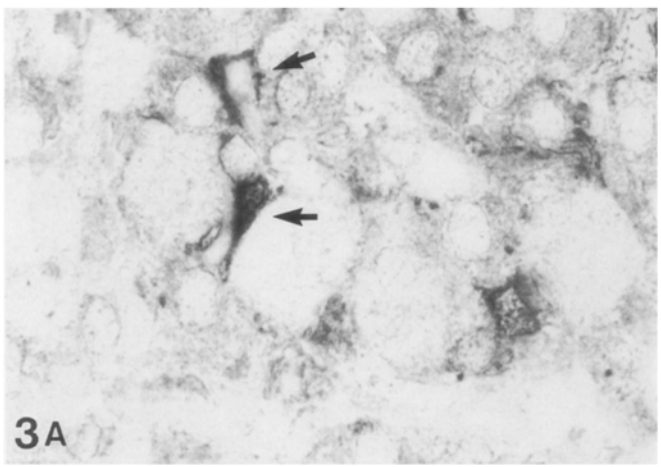

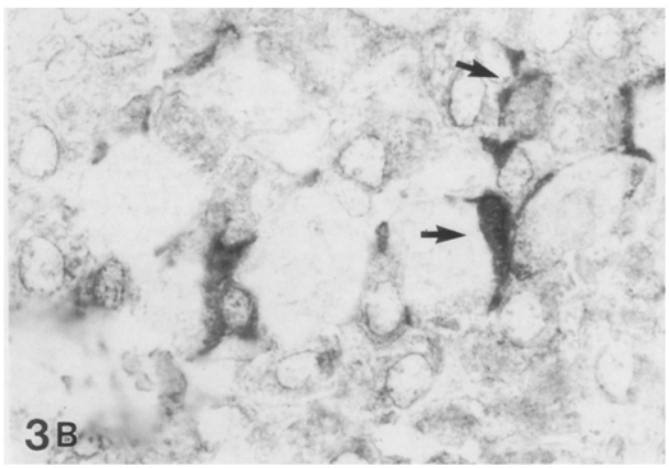

Fig. 1. A Distribution of fibronectin in the rat anterior pituitary. *Arrow* indicates marginal layer of pars intermedia. **B** Higher magnification of the tissue section immunolocalized for fibronectin. **A** ×180; **B** ×920

Fig. 2A and B. Two consecutive sections of rat anterior pituitary immunostained with fibronectin antiserum (**A**) and with S-100 protein antiserum (**B**). Note that the section immunostained for S-100 protein, in spite of pretreatment with H_2O_2, still shows background reaction (*asterisk*). ×360

Fig. 3A and B. Adjacent mirror sections incubated with fibronectin antiserum (**A**) and with S-100 protein antiserum (**B**). *Arrows* indicate the same cells. ×920

not influenced by preabsorption of the first antibody with heterogenous antigen (data not shown).

Discussion

We demonstrated for the first time the localization of fibronectin in the rat anterior pituitary. The fibronectin-immunoreactive cells were stellate in shape. In the consecutive mirror sections, fibronectin immunoreactivity was found to coexist with S-100 protein immunoreactivity, whereas the latter has been known to be specifically detected in the FS cells (Nakajima et al. 1980). We therefore conclude that the cells immunolabeled for fibronectin are nothing but FS cells of the anterior pituitary.

The double bridge PAP method (Vacca et al. 1980) was adopted for the immunolocalization of fibronectin in the present study. This method was highly sensitive as compared with the ordinary PAP method of Sternberger (1974), though relatively weak immunoproduct could be visualized by the ordinary technique in our preliminary experiment. The same method was also used for the immunostaining of S-100 protein. As the result, the immunoreactivity was increased without much change in background intensity.

Fibronectin is large glycoprotein that can be synthesized by a wide variety of cell types including fibroblast and certain epithelial cells (Hynes and Yamada 1982). The fibronectin is present in soluble form in the plasma and in insoluble form in the extracellular matrices (ECM) (Yamada 1983) and within the cytoplasm of fibronectin producing cells (Sabet and Gordon 1987). The present study revealed the appearance of fibronectin only in the FS cells and not in the ECM, probably reflecting the quantitative differences in the fibronectin present.

Fibronectin has been implicated in a variety of biological activities, such as cell anchorage, polarity, migration, phagocytosis, differentiation and proliferation (Ruoslahti 1988). And it has been demonstrated that the biological functions are mediated by many binding sites in the fibronectin polypeptide. Although the exact role of fibronectin in the rat anterior pituitary is unknown, the present findings suggest a novel biological mechanism by which FS cells would participate in the regulation of pituitary function through the interaction of fibronectin with hormone secreting cells.

An immunoelectron microscopic study now in progress will provide more evidence on the ultrastructural location of fibronectin in the FS cells and on the cellular interaction between hormone secreting cells and FS cells.

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