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Immunohistochemical Localization of S-100 Protein in the Pig Pituitary Gland

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With 4 figures

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Summary

Immunohistochemical localization of S-100 protein was studied in anterior, intermediate and posterior lobe of the pig pituitary gland. Two immunopositive cells for S-100 protein were identified: the folliculo-stellate cells (FSc) in the glandular lobes and the pituicytes in the neural lobe. In the anterior lobe, immunoreactive folliculo-stellate cells were scattered among secretory cells. In the area where the secretory cells form strands and follicle-like groups the positive cells were concentrated in groups. In the intermediate lobe, S-100 protein-positive cells were located sparsely among secretory cells and next to secretory follicles and the pituitary cleft. These FSc were more voluminous and displayed fewer cytoplasmic processes. In the neurohypophysis, positive reaction for S-100 protein was seen in the pituicytes. These cells were distributed singly or concentrated in groups. The distribution, and morphologic characteristics of the FSc in the glandular lobes and the pituicytes in the neural lobe in the pig indicate different origin of both S-100 protein-positive cells.

Introduction

Rinehart and Farquhar (1953) and Farquhar (1957) described the presence of non-chromophilic cells in the rat adenohypophysis. These types of cells were later identified in other species (Carlon, 1967) and were referred to as folliculo-stellate cells (Vila-Porcile, 1972). Ultrastructural studies demonstrated various fine structural properties on them (Takei et al., 1980; Girod et al., 1985; Kurosumi, 1985; Girod and Lheritier, 1986; Lloyd and Mailloux, 1988; Kameda, 1996). The localization of folliculo-stellate cells (FSc) in the glandular lobes was described to be similar in the human and animal species (Höfler et al., 1984; Morris and Hitchock, 1985; Marín et al., 1989).

The FSc of pituitary gland are morphologically characterized by a stellate appearance and an elongated cell processes. By immunohistochemistry, the pituitary FSc exhibit immunoreactivity for several different proteins: S-100 protein, glial fibrillary acid protein, vimentin, and cytokeratines. Several reports have revealed expression of the S-100 protein in the FSc in the hypophysis of various animal species and human (Nakajima et al., 1980; Takahashi et al., 1984; Girod et al., 1986; Tachibana and Yamashima, 1988; Kameda, 1991; Tsuchida et al., 1991; Watanabe and Hashimoto, 1993; Soji et al., 1994). However, the immunoreactivity was not detected in FSc in the anterior pituitary in the goat (Shirasawa et al., 1984). The aim of present study was to localize S-100 protein in three lobes of the pig pituitary gland.

Material and Methods

The study was performed on five adult pigs killed at the slaughterhouse. The samples of hypophyses were fixed in 0.1 M phosphate buffered 4% formaldehyde for 24 h at room temperature, dehydrated and embedded in paraffin. Serial sections were cut at 5 μm thickness, mounted on slides coated with poly-L-lysine and stained with haematoxylin-eosin or processed following the avidin-biotin-peroxidase complex (ABC) method (Hsu et al., 1981). Following deparaffinization, sections were hydrated, incubated for 20 min in 0.3% H_2O_2 in phosphate-balanced salt solution (PBS) to reduce endogenous activity, pre-incubated with 2% goat serum to mask unspecific binding sites, incubated overnight with the first polyclonal rabbit anti-S-100 protein antibody (Immunotech, s.r.o., Bratislava, Slovakia), diluted 1:100. After washing in PBS the slides were incubated with goat anti-rabbit biotinylated immunoglobulin (Vector Laboratories, Burlingame, CA, USA) at 1 : 200 dilution. After 1 h of incubation, the sections were incubated with ABC (Vectastain ABC kit; Vector, Burlingame, CA, USA) and developed with 0.05% 3′.3′-diaminobenzidine (DAB) and 0.03% v/v H_2O_2. Some sections were counterstained with Mayer's haematoxylin. Negative controls were performed by omitting the primary antibody.

Results

The S-100 positive cells in the pars distalis of the pig hypophysis were scattered predominantly among the immunonegative glandular secretory cells (Fig. 1). Reactive cells have no signs of secretory cells but clearly a stellate shape with long extending processes. Their slender cytoplasmic processes, strongly positive for S-100 protein, were seen to penetrate among the secretory cells suggesting the folliculo-stellate shape. Immunoreactivity for S-100 protein was observed in the cytoplasm (Fig. 2). In the secretory cells no positive reaction in the nucleus and surrounding cytoplasm was observed. The positive cells were distributed irregularly throughout the distal lobe and no preferential localization of S-100 protein immunoreactivity was found. S-100 protein positive cells were gathered only in the place of accumulations of secretory cells into strands and follicles, where they lined one side of secretory cells.

In the intermediate lobe, the distribution of positive cells were similar to that in the distal lobe. The S-100 protein-positive cells were located between immuno-negative glandular secretory cells and were distributed sparsely throughout the intermediate lobe (Fig. 3). No preferential localization for S-100 protein cells was observed, except for the area of

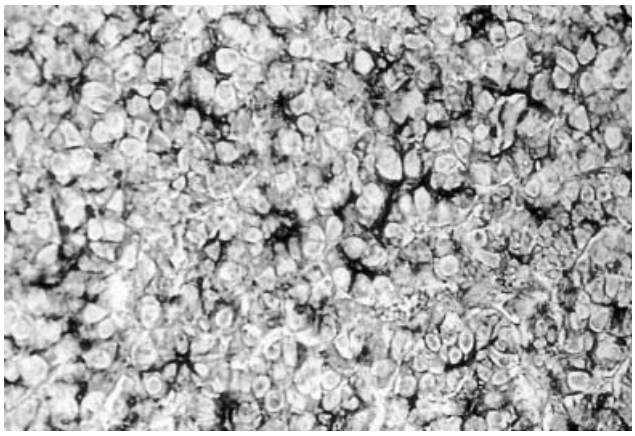

Fig. 1. Section of pars distalis. Folliculo-stellate cells reactive to S-100 protein with long processes entering among the secretory cells. ×300.

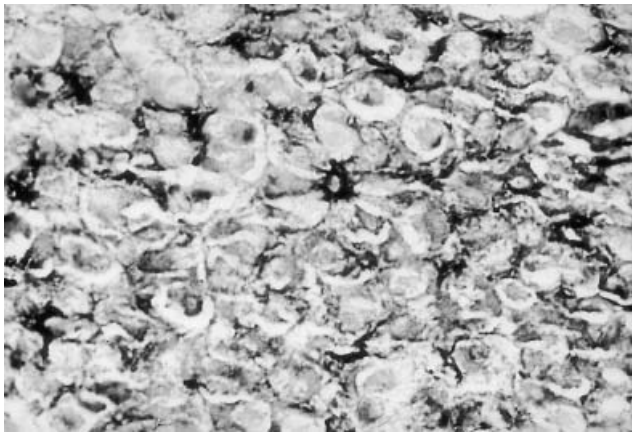

Fig. 2. Section of pars distalis. Immunoreactivity to S-100 protein was observed in the cytoplasm of folliculo-stellate cells. ×400.

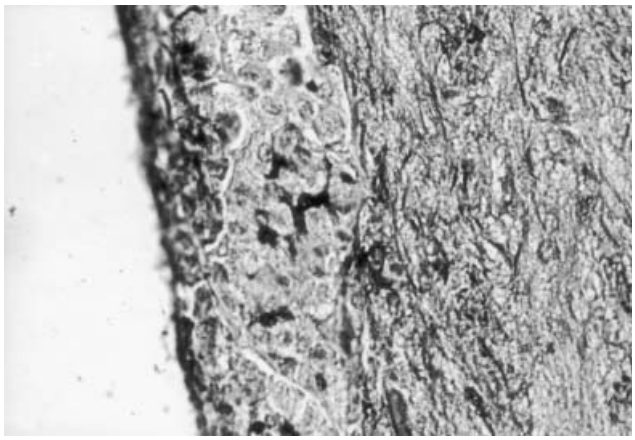

Fig. 3. Section of pars intermedia and adjacent neurohypophysis. In the pars intermedia reactive folliculo-stellate cells to S-100 protein are irregularly distributed among the secretory cells. ×600.

secretory follicles. Some of the positive cells were lining the pituitary cleft. The positive cells displayed less cytoplasmic processes and their bodies were more voluminous than those in the distal lobe. The dense network of cell processes encircling secretory cells, as seen in the pars distalis was not observed in the intermediate lobe. The secretory cells were immunonegative to S-100 protein.

In the neurohypophysis, S-100 protein was expressed in the supporting cells – the pituicytes. The morphologic features of the positive cells were different form those of the glandular lobe. These cells were more elongated with one or two cytoplasmic processes. The positive cells displayed more spindle-like shape when compared with the folliculo-stellate shape of the glandular lobe. In comparison with the anterior lobe, they were less numerous and were distributed individually or concentrated in higher number (Fig. 4). A strong reaction to S-100 protein was seen in the cytoplasm of pituicytes.

Discussion

Expression of S-100 protein in animals as well as in the human hypophysis has been reported principally in the FSc. Their distribution in the anterior lobe was reported to be uniform. In the human pituitary gland (Marín et al., 1989) the cells with S-100 protein were localized in both the central and the peripheral region of this lobe. In the pig no preferential localization of S-100 protein was found. Differences in localization of S-100 protein positive FSc, related to the stage of development were described in the rat hypophysis (Soji et al., 1994). In the distal lobe of the pig pituitary gland the FSc were distributed irregularly and no significant predilection in their distribution was found. An immunoreaction for S-100 protein was observed in the cytoplasm. In the rat pituitary gland, a diffuse immunoreaction for S-100 protein was observed in both the cytoplasm and nucleus (Tsuchida et al., 1991) or only in the nucleus (Amano et al., 1993).

Marín et al. (1989) suggested the existence of different types of FSc cells after using GFAP, vimentin and S-100 protein. Orgnero de Gaisan et al. (1993) described two types of S-100 protein-positive cells: one forming the clusters of two to four cells with slight immunoreactivity and the other type consisting of isolated cells with a stellate profile and stronger reaction to the S-100 protein. The reactive FSc observed in the area of secretory strands and follicles may signify their

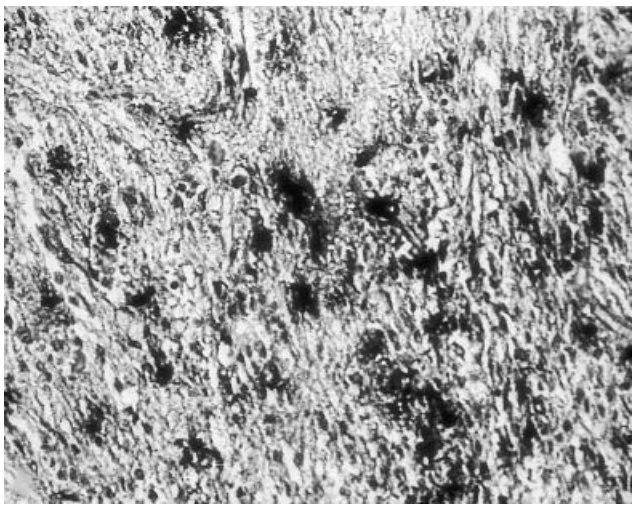

Fig. 4. Section of neural lobe. A spindle-shape pituicytes reactive to S-100 protein are seen among axons. ×600.

different functional role rather than the existence of more than two types of the FSc described by the above mentioned authors.

As in the distal lobe, also in the intermediate lobe the distribution of S-100 protein positive cells was reported to be not uniform. In the rat hypophysis, the S-100 protein positive cells were the marginal cells lining pituitary cleft (Amano et al., 1993). In the cat, some S-100 protein-immunoreactive cell bodies, or their processes, were found lining the lumen of the large cavities within the intermediate lobe (Marín and Boya, 1991). Also in the pig we found a reactive product lining the follicular cavities and some positive cells were lining the pituitary cleft. In the intermediate lobe, most of the S-100 protein-positive FSc showed characteristics similar to those of the distal lobe. The differences in the shape of FSc found in the intermediate lobe are in our opinion of functional significance.

In the neurohypophysis, the presence of S-100 protein was observed in the pituicytes. In the pituicytes, like in the FSc of both glandular lobes, the positive reaction predominanted in the cytoplasm. The concentration of pituicytes in some areas is probably accidental and/or may be related to the accumulation of axons.

The FSc have specific characteristics predominating being the absence of specific secretory granules and presence of long cytoplasmic processes between the granulated cells. These properties led to the hypothesis that FSc may belong to the neuroectodermal cells. The ultrastructural similarity of the FSc to the marginal cells of Rathke's cleft (Soji et al., 1989) suggests that these cells are derived from Rathke's pouch, as are other secretory cells in the anterior pituitary. The immunopositivity for S-100 protein (Cocchia and Miani, 1980; Nakajima et al., 1980) and GFAP (Velasco et al., 1982), and vimentin (Marín et al., 1989) in these cells indicates their glial origin (de Bold et al., 1980). According to some authors who demonstrated the S-100 protein immunoreactive cells in the intermediate lobe of the rat and Mongolian gerbil these cells probably correspond to pituicytes emigrated to this area (Marín et al., 1989). Marín and Boya (1991) found no evidence of neurohypohyseal cell invasion to the glandular parenchyma in the cat pituitary gland. Moreover, the authors demonstrated the presence of immature interstitial cells, which form clusters within the pars intermedia separated from the neural lobe by a thick connective tissue. No spatial relationship between the neurophyseal supporting cells and glandular FSc were found in the pig. This finding and the different morphological and structural properties and also different embryonal development of adenohypophysis and neurohypophysis exclude their common origin.

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