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Immunocytochemical study of the epithelial lining of naturally occurring cysts in the rat intermediate lobe

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Summary. An immunocytochemical study of the epithelial lining of naturally occurring cysts in the rat intermediate lobe (IL) has been carried out. Paraffin-embedded sections, in which cysts were identifiable, were treated either with anti-serotonin or anti-S-100 protein sera. S-100-positive cells were intermingled with glandular cells surrounding the cyst lumen. These S-100-positive cells sent slender cytoplasmic processes as if to cover the apical surface of neighbouring cells. Rarely were 5-HT-immunopositive cells seen in the cyst epithelial lining. Most cells of the marginal layer of the IL were found reactive either to an S-100 or a-5-HT serum. The presence of an epithelial lining positive to S-100 protein sera is in keeping with the notion that cysts in the IL might form as evaginations of the epithelial lining of the pituitary cleft. The lack of correspondence between 5-HT-positive cells in the marginal layer and the cyst lining is controversial. A peculiar spatial relationship of 5-HT cells with the vascular network of the IL is suggested.

Key words: Pituitary, Intermediate Lobe, Cysts, Immunocytochemistry, Rat (Sprague-Dawley)

Introduction

Classification of pituitary cysts is difficult and inconsistent (reviewed by Benjamin, 1981). Unequivocal evidence concerning the origin and significance of cysts in the pituitary gland is still lacking. However, at least methodologically, pituitary cysts might be divided into naturally occuring and experimentally-induced cysts. Redecker (1989) has reported that naturally-occurring cysts in the intermediate lobe (IL) of the pituitary of the gerbil are mainly lined by cells which show no reaction to the anti-MSH serum and have morphological features similar to those of folliculo-stellate and marginal cells. His findings are in keeping with the notion, suggested earlier by others (Guizzetti, 1927; Howe and Thody, 1968), that evagination of epithelial-lined tubes from the posterior wall of Rathke's cleft are the origin of some of the spontaneously arising cysts in the IL of the pituitary. If this were the case, the epithelium lining the cyst, if any, should have cytological and immunocytochemical features similar to those of the cells in the marginal layer. At present, there are specific markers for folliculo-stellate cells and cells lining the pituitary cleft. These cells express the brain-specific S-100 protein (Cocchia and Miani, 1980; Shirasawa et al., 1983). Furthermore, marginal layer cells, as well as a polimorphous group of cells scattered throughout the rat IL were found reactive to an anti-serotonin (5-HT) serum by Carvajal et al. (1989).

This work has been carried out to characterize the lining of naturally-occurring cysts in the IL of the rat pituitary immunocytochemically using anti-S-100 protein and anti-serotonin (5-HT) sera. Comparison of cytological and immunohistochemical features of the cyst epithelial lining with those of the marginal layer cells might help to gain insight into the origin of cysts in the rat IL.

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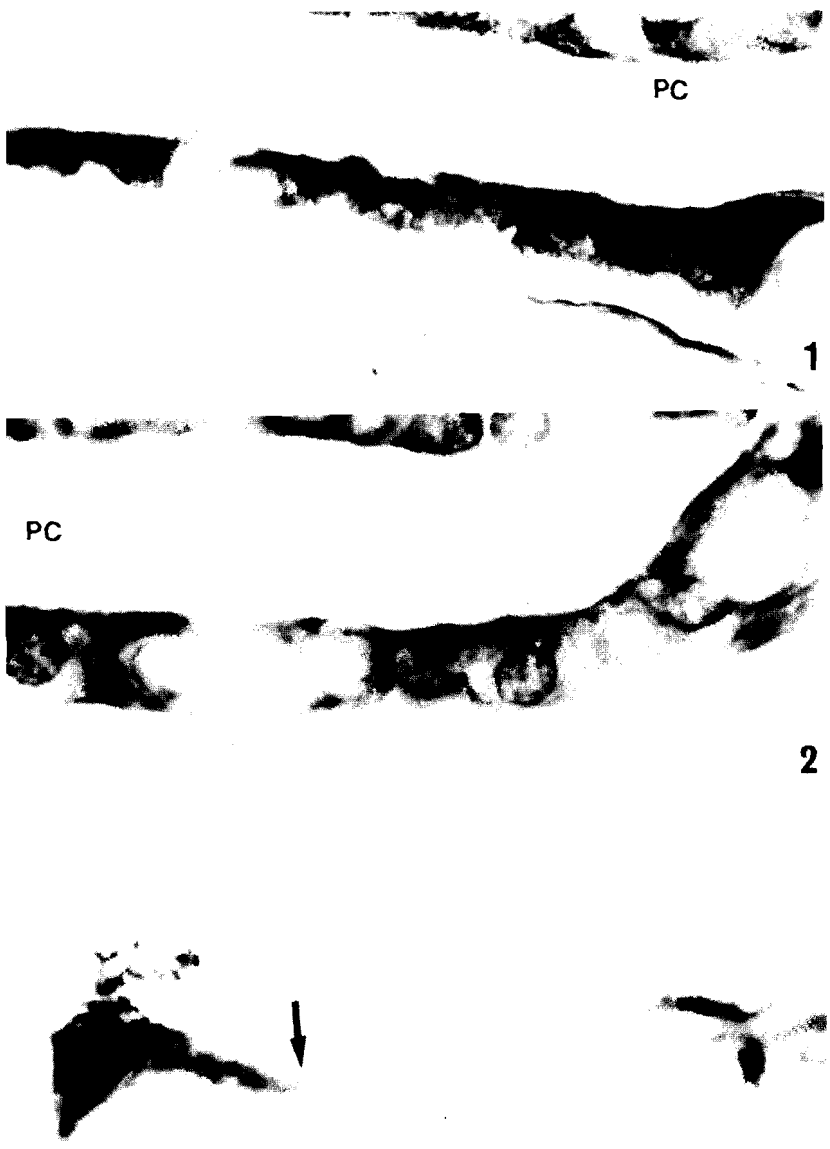
Materials and methods

Adult Sprague-Dawley rats (200-220 g body weight) were used throughout. After ether anaesthesia 20 males and 20 females were fixed by intracardiac perfusion with Bouin-Hollande's solution and postfixed in the same solution for three days. After embedding in paraffin wax the pituitaries were cut in the sagital plane and 5 μm-thick sections mounted on glass slides.

Sections were scanned with a light microscope to detect cysts in the IL and, subsequently, deparaffinized and stained using the peroxidase-antiperoxidase (PAP) method (Sternberger et al., 1970). Serial sections were



Figs. 1 and 2. Cells of the marginal layer of the IL reactive to an S-100. **(Fig. 1)** and a 5-HT sera. **(Fig. 2).** Immunonegative cells are observed intermingled with reactive cells in both case. PC (pituitary cleft). × 4,800

Fig. 3. Cell immunoreactive to an S-100 protein sera in the luminal wall of a cyst. A long, slender cytoplasmic process of this cell (arrow) covers the apical surface of neighbouring cells trying to contact a process from another S-100 positive cell. × 4,800

incubated overnight either with antibody to 5-HT (1:800) (Chemicon International Inc.) or with antibody to S-100 protein (1:800) (DAKO). Irrespective of the first antisera used, sections were incubated further with anti-rabbit IgG (1:100, 60 min) (Dako) and PAP (1:100, 60 min) (Dako). The final reaction product was visualized with 3-3'-diaminobenzidine tetrahydrochloride.

Samples were studied and photographed with a Leitz-dialux EB 20 light microscope.

Results

Naturally-occurring cysts in the rat IL were not a frequent finding. Cysts were seen in only 3 of the 40 animals studied.

Distribution of immunoreactive cells, both in cysts

Fig. 4. A narrow, irregular duct is seen connecting the marginal layer (ML) and a cyst (C). Fine strands immunostained with an S-100 protein serum are seen in the duct. × 1,500

Fig. 5. A fusiform cell reactive to an S-100 protein serum is seen in contact with an interlobular wall, sending a long cytoplasmic process towards a cyst in the vicinity. × 4,800

and marginal layer, was consistent in the three animals studied. In all cases, S-100 protein-positive cells outnumbered those reactive to a 5-HT serum.

Most cells of the marginal layer of the IL were found reactive either to an S-100 or a 5-HT serum (Figs. 1, 2). Occasionally, nonreactive cells (glandular cells) were found among immunopositive cells, facing the pituitary cleft. On the contrary, cysts did not show a distinct lining of immunoreactive cells. S-100 protein-positive cells were intermingled with glandular (immunonegative) cells surrounding the cyst lumen. These S-100-positive cells sent slender cytoplasmic processes as if to cover the apical surface of neighbouring cells separating them from the cyst lumen (Fig. 3). 5-HT-immunopositive cells were infrequently seen in the cyst epithelial lining.

Some sections revealed a continuity between the cyst lumen and pituitary cleft through narrow and tortuous ducts lined both by glandular cells and thin strands showing S-100 protein immunoreaction (Fig. 4).

S-100 protein and 5-HT immunoreactive cells were found interspersed among glandular cells in the IL. Triangular or fusiform cells reactive to an S-100 protein were seen in close contact with interlobular walls. Occasionally, these cells sent a long cytoplasmic process towards a neighbouring cyst (Fig. 5). Some of the 5-HT-positive cells resembled folliculo-stellate cells.

Discussion

Several different conditions have been said to contribute to the genesis of cysts in the pituitary gland. Captivity (Hanström, 1947, 1948, 1952; Robertson and Weler, 1962) and domesticity (Oboussier, 1948; Wingstrand, 1951), stress (Abel et al., 1971), puberty and gestation (Vázquez and Amat, 1968), castration and thyroidectomy (Vázquez and Amat, 1968) and aging (Yoshie and Honma, 1978) have been shown to increase the incidence of cyts in the pituitary gland. However, unequivocal evidence concerning the origin of these cysts is still lacking.

Folliculo-stellate (FS) cells and marginal layer cells share morphological and immunocytochemical features (Dingemans and Feltkamp, 1972; Vila-Porcile, 1973; Shirasawa et al., 1983), but they differ according to their localization within the pituitary gland. Marginal

cells are found along Rathke's cleft while FS cells can be found throughout the pituitary gland, lining small follicles (Dingemans and Feltkamp, 1972). It has been proposed that marginal cells and FS cells form a continuous cavity system in the pituitary gland (Dingemans and Feltkamp, 1972; Vila-Porcile, 1973; Ciocca and González, 1978). With regard to this, the pituitary cleft might be considered as a great dilation of this common canalicular system and, similarly, cysts might be considered as enlarged follicles. The common morphological features of FS and marginal cells earlier described by Guizzetti (1927) and Romeis (1940) and their relationship as constituents of the lining of the pituitary canalicular system, prompted Dingemans and Feltkamp (1972) and Redecker (1989) to suggest that FS cells might derive from marginal cells. This in keeping with the notion that cysts in the pituitary gland might form as evaginations of the epithelial lining of the pituitary cleft, as stated by Howe and Thody (1968) and Gon et al. (1987).

The narrow and tortuous communications between the pituitary cleft and cyst lumen reported herewith provide further evidence for the hypothesis of cell migration from the posterior wall of Rathke's cleft to form IL-cysts. Connections between cyst lumen and pituitary cleft may disappear at later stages of the cyst development (Benjamin, 1981) obscuring the origin of the new-formed cavity. However, in our experience serial sections never failed to show communications between the cyst and the pituitary cleft. As a reminder of their putative origin from the marginal layer, some cells along the cyst lumen showed S-100 protein immunoreaction. Noticeably, the histological aspect of the cyst lining and that of the marginal layer were different. Marginal cells were almost always present as a single, flattened layer of S-100-positive cells surrounding the pituitary cleft. On the contrary, S-100-positive cells were not so abundant in the cyst lining. However, despite their relatively small numbers, S-100-positive cells coated most of the luminal surface of the cyst by sending slender cytoplasmic processes that covered the apical surface of neighbouring glandular cells. Because of its particular structure the epithelial lining of the cyst may be easily neglected, as already pointed out by Benjamin (1981).

As opposed to the obvious presence of S-100 protein-labelled cells in the epithelial lining of the cyst, 5-HT cells were rarely seen. This is a controversial finding. As stated above, most marginal layer cells were stained either with an S-100 or a 5-HT serum. A similar pattern of S-100 and 5-HT immunoreactive cells might be expected in the cyst lining if cell migration from the marginal layer is to be considered as the origin of these cavities in the IL. The process of selective migration of S-100-positive cells from the marginal layer to the cyst wall seems hardly possible. Nerve fibres and terminals stained with 5-HT antiserum have been seen in the rat IL by several authors (Westlund and Childs, 1982; Friedman et al., 1983; Payette et al., 1985). In this study, cell bodies containing 5-HT in the IL have also been identified, as previously reported by Carvajal et al. (1989). At present there is no definitive data on the origin of 5-HT-positive cells in the rat IL. It is not known whether 5-HT labelled cells produce and release 5-HT themselves or, on the contrary, they take in 5-HT from the surrounding media. To give an appropriate answer to this question further in vitro studies are needed. However, the peculiar localization of 5-HT-labelled cells enables a reasonable speculation about the source of 5-HT. Carvajal et al. (1989) described that some 5-HT labelled cells had a stellate shape and conspicuous cytoplasmic processes resembling folliculo-stellate cells, hence suggesting that some of the 5-HT-labelled cells might be regarded as FS cells. We have now seen that most 5-HT-labelled cells are found in the marginal layer, some in close contact
with the interlobular spaces and, only a few, spread throughout the IL. This peculiar distribution is not consistent with the distribution of 5-HT nerve fibres reported by different authors (Westlund and Childs, 1982; Friedman, 1983; Payette et al., 1985) so it would be unlikely that 5-HT-positive cells took in 5-HT from nerve terminals. On the other hand, the distribution of 5-HT cells in the IL matches the distribution of the vascular network of the IL. It is well known that the IL has a limited vascular supply; however, a fine capillary meshwork has been described in the ventral surface of the IL, close to the pituitary cleft (Murakami et al., 1985; Carbajo-Pérez et al., 1989). This ventral network is fed by capillary loops detached from a dense vascular network in the ventral surface of the neural lobe in close contact with the IL (Carbajo-Pérez et al., 1989). It is tempting to suggest that marginal cells and FS cells in the IL have the ability to take in 5-HT conveyed through the capillary network. This would explain the absence of 5-HT immunoreaction in the cyst lining. When marginal cells migrate to deeper locations in the IL, they lose contact with the capillary network, and hence with the source of 5-HT. The functional meaning of the presence of 5-HT in some of the marginal cells and of FS cells remains within the bounds of speculation, as do many of the functions of these cells suggested by several authors (Salazar, 1968; Cardell, 1969; Forbes, 1972; Shiotani, 1980; Nunez et al., 1985; Allaerts, 1989).

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