

ペプチド(ガラニン,PACAP27,PACAP38)含有神経によるニ ワトリハーダー腺の神経支配に関する免疫組織化学的研究

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Immunohistochemical Study on the Innervation of the Chicken Harderian Gland by Peptides (Galanin, PACAP27 and PACAP38) -containing Nerves

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Localization of neuropeptides (galanin, PACAP27 and 38) was investigated in the chicken Harderian gland by immunohistochemistry. Immunoreactivity for three peptides examined in this study was detected on nerve elements in the pterygopalatine ganglion and the gland. Pterygopalatine ganglion contained nerve cell bodies showing immunoreactivity for galanin, PACAP27 and 38. Dot-form immunoreactivity for PACAPs was also detected around immunonegative nerve cell bodies. Galanin immunoreactivity was observed on larger nerve cell bodies and those were most commonly observed in the ganglion. Nerve fibers showing immunoreactivity for the neuropeptides were distributed in the interstitial tissue of the Harderian gland. Fine immunoreactive nerve fibers were found running beneath the capsule and between acini. No immunoreactive nerve fibers were found contacting with epithelial cells of the collecting duct. The present data suggested that nerves containing galanin and PACAPs have a relation to the regulation of the immune function of the chicken Harderian gland..

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Key words : innervation, Harderian gland, neuropeptides, immunohistochemistry

Introduction

The chicken Harderian gland is the dominant orbital gland. The gland is a compound tubulo-acinar structure, and its secretory duct opens into the inferomedial portion of the conjunctival sac deep to the nictitating membrane (WIGHT *et al.*, 1971 a ; BURNS, 1976 a). The gland has two important functions : production of a secretion that lubricates the nictitating membrane (WIGHT *et al.*, 1971 b), and protection of the oculonasal region from exogenous antigens (BURNS, 1976 b ; OLÁH *et al.*, 1996). The aggregation of plasma cells is observed in the interstitial tissue of the chicken Harderian gland (MUELLER *et al.*, 1971 ; ALBINI *et al.*, 1974). The cells are rich in immunoglobulins, Ig A, Ig G and Ig M (ALBINI *et al.*, 1974). Thus the chicken Harderian gland may contribute in an important component to the local immune system in the oculonasal region (MUELLER *et al.*, 1971).

A rich supply of autonomic nerves to the Harderian gland can be recognized in birds. Histochemical studies showed the innervation of the gland by catecholamine-

containing and AChE-positive nerves (FOURMAN and BALLANTYNE, 1967 ; WALCOTT *et al.*, 1984 ; WALCOTT and MCLEAN, 1985). Immunohistochemical study by WALCOTT and his coworkers (1985, 1989) reported that many nerve fibers showing immunoreactivity for neuropeptides (vasoactive intestinal polypeptide ; VIP and substance P ; SP) were seen among the plasma cells. It is supposed that neuropeptides play an important role in the regulation of the immune function of the chicken Harderian gland. Galanin is widely distributed in the peripheral nervous system (cf. HIRAMATSU and OHSHIMA, 1995). Pituitary adenylate cyclase-activating peptide (PACAP) is a neuropeptide of secretin/glucagon/VIP family and two molecular forms are known, PACAP 27 and PACAP 38. Both forms of this peptide were also contained in the peripheral nervous system (cf. ARIMURA, 1992). But the distribution of nerves containing these neuropeptides has never been known in the chicken Harderian gland.

In the present study, we aim at the elucidation of the distribution of neuropeptides (galanin, PACAP 27 and 38)-containing nerve elements in the chicken Harderian gland and the pterygopalatine ganglion. The pterygopalatine ganglion is located along the superior margin of the Harderian gland and supplies postganglionic nerve fibers to the gland (GIENC and ZABOREK, 1984). Moreover the neural regulation of the local immune system in the chicken should be discussed.

Materials and Methods

Six adult male White Leghorn chickens weighing 1.5-2.5 kg were used in this study. Chickens were perfused with the mixture of 4% paraformaldehyde, 0.1% glutaraldehyde and 0.21% picric acid in 0.1 M phosphate buffer (pH 7.6) following 0.75% sodium chloride solution under the anesthesia with sodium pentobarbital. Harderian glands were immediately removed from an orbital cave of both sides and each gland was cut in three tissue blocks with a razor blade. Tissue blocks were immersed in the fresh same fixative at 4°C for 6 hours and transferred into 20% phosphate-buffered sucrose for cryoprotection. And then frozen sections at 15 μ m thickness were made in a cryostat in the conventional manner.

Streptavidin-biotin method (GUESDON *et al.*, 1979) was applied on frozen sections according to the way previously described (HIRAMATSU and OHSHIMA, 1995). Rabbit antisera against natural pig galanin (Chemicon, USA, AB1985, diluted to 1 : 1000), PACAP 27-NH₂ and PACAP 38 (Peninsula, USA, IHC 8922 and IHC 8920, 1 : 2000) were used as primary antibodies in this study.

For controls, some sections were incubated with antisera which were preabsorbed with 10 μ g/ml each peptide at 4°C for 24 hours.

Results

Immunoreactivity for galanin and PACAPs was observed on the nerve elements in the pterygopalatine ganglion and the Harderian gland. Results of the control examination showed negative staining.

The pterygopalatine ganglion contained many nerve cell bodies showing immunoreactivity for neuropeptides. Galanin-immunoreactive nerve cells were fre-

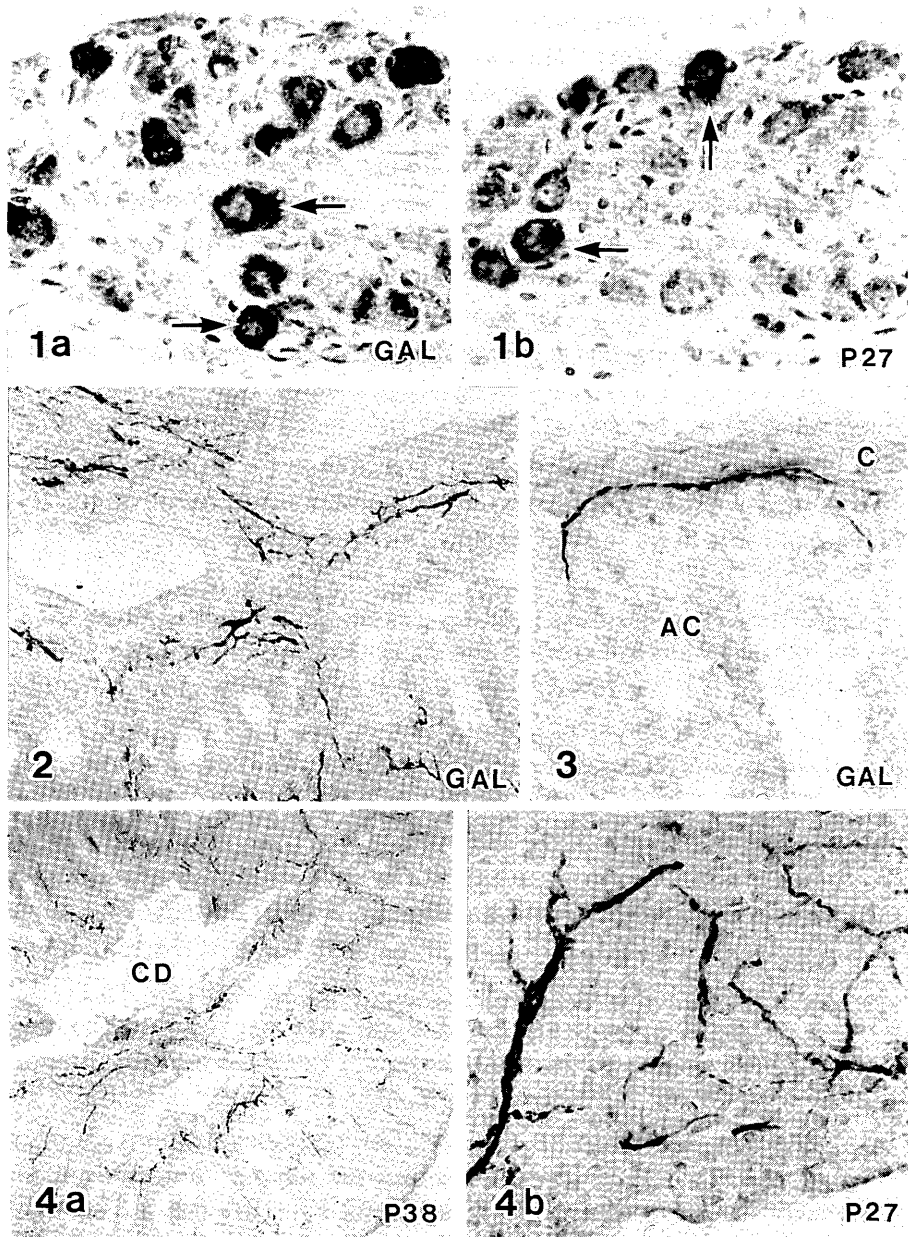


Fig. 1. Immunoreactive nerve cells in the pterygopalatine ganglion. Nerve cell bodies showing immunoreactivity for galanin (a) and PACAP27 (b) (arrows) are observed in the ganglion. $\times 400$ (a, b).

Fig. 2. Galanin-immunoreactive nerve fibers are distributed in the interstitial tissue of the chicken Harderian gland. $\times 185$.

Fig. 3. Fine nerve fiber showing immunoreactivity for galanin is found running beneath the capsule (C) but not in the acinus (AC) of the gland. $\times 460$.

Fig. 4. (a) Low magnification view of the Harderian gland. Many nerve fibers showing PACAP38 immunoreactivity are distributed in the interstitial tissue. CD: collecting duct $\times 75$. (b) High magnification view of the interstitial tissue. Dense network of PACAP27-immunoreactive nerve fibers is observed. $\times 370$.

quently found in the ganglion (Fig. 1 a). These ganglion cells were relatively large in size. Ganglion cells showing PACAP27 immunoreactivity were also found but the number of them was less than galanin-immunoreactive ones (Fig. 1 b). A small number of ganglion cells showed PACAP38 immunoreactivity. Dot-form immunoreactivity for PACAPs were detected around immunonegative ganglion cells.

Galanin-immunoreactive nerve fibers were distributed throughout the entire gland. Dense network of galanin-immunoreactive nerve fibers was found in the interstitial tissue (Fig. 2). The network was formed in the deep part of the interstitial tissue of the Harderian gland and no nerve fibers contacted with epithelial cells of the collecting duct. Fine nerve fibers showing galanin-immunoreactivity were observed in the capsule of the gland (Fig. 3).

Immunoreactivity for PACAPs showed the similar distribution pattern with galanin immunoreactivity. PACAP-immunoreactive nerve fibers were mainly distributed in the interstitial tissue and made dense network (Fig. 4 a, b). In the acini we could not find nerve fibers showing immunoreactivity for any neuropeptides (Fig. 3).

Discussion

In the present study we clarified the distribution pattern of nerves containing neuropeptides, galanin and PACAPs, in the chicken Harderian gland. The gland is considered as the local immune tissue at the oculonasal region (MUELLER *et al.*, 1971) because of the accumulation of plasma cells in its interstitial tissue (WIGHT *et al.*, 1971 a ; BURNS, 1975 ; NIEDORF and WOLTERS, 1978). These plasma cells produce and secrete immunoglobulins in response to the local antigen stimulus (ALBINI *et al.*, 1974 ; BURNS, 1976b ; OLÁH *et al.*, 1996). Recently, many studies have shown the interactive actions between the nervous system and the immune system. In particular, neuropeptides might play an important role in the regulation of immune system functions (BELLINGER *et al.*, 1990). WALCOTT and his colleagues (1985, 1989) have shown the peptidergic innervation of the Harderian gland. They detected nerve fibers showing immunoreactivity for neuropeptides such as VIP and SP in the interstitial tissue and suggested the peptidergic regulation of the immune functions of the gland.

We found the dense network of nerve fibers showing immunoreactivity for galanin and PACAPs in the interstitial tissue of the chicken Harderian gland. Our previous study showed the nerve endings containing a few large dense-cored vesicles which indicate the peptidergic feature and contacted with plasma cells in the interstitial tissue (HIRAMATSU and OHSHIMA, 1998). These data suggest the regulation of plasma cells by peptidergic nerves which may release galanin and PACAPs. Some studies (GARRIDO *et al.*, 1996 ; MARTINEZ *et al.*, 1998) reported the participation of PACAP in controlling local immune responses. The immunological action of galanin has been unknown. But galanin-immunoreactive nerves were found in the cortex of the Bursa Fabricii and their link with B cells was suggested (ZENTEL and WEIHE, 1991). So it is possible that galanin and PACAPs play a role in the control of the immune function of the chicken Harderian gland.

The pterygopalatine ganglion supplies the post ganglionic nerve fibers to the

Harderian gland (GIENC and ZABOREK, 1984). Dot-form immunoreactivity for PACAPs was found around immunonegative cell bodies in the ganglion. Nerve terminal containing many large dense-cored vesicles was found contacting a ganglion cell (HIRAMATSU and OHSHIMA, 1998). PACAPs might also have an indirect influence to functions of the gland through the ganglion cells.

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ペプチド（ガラニン，PACAP 27，PACAP 38）含有神経による ニワトリハーダー腺の神経支配に関する免疫組織化学的研究

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ニワトリハーダー腺内における神経ペプチド（ガラニン，PACAP 27 および 38）の局在について，免疫組織化学的手法を用いて研究した。検索した神経ペプチドに対する免疫陽性反応は，翼口蓋神経節と腺内の神経要素に観察された。翼口蓋神経節には，ガラニン，PACAP 27 及び 38 に免疫陽性反応を示す細胞体が含まれていた。ガラニン免疫陽性細胞は，比較的大型の細胞でもっとも多く観察された。免疫陰性細胞体の周囲に PACAP に対する免疫陽性反応が点状に観察された。ハーダー腺において神経ペプチドに免疫陽性反応を示す神経線維は，間

質に分布していた。これらの陽性線維は，密な網目状構造を形成していた。しばしば被膜下から腺房間を走行する細かいペプチド含有神経線維が観察された。導管上皮に接する神経線維は認められなかった。本研究の結果は，ガラニン及び PACAP を含有する神経がニワトリハーダー腺の免疫機能の調節に関わっていることを示唆する。

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