LEPTIN EXPRESSION
IN GOOSE (ANSER ANSER) PREEN GLANDS

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Abstract

The aim of this study was to investigate the presence of leptin in healthy goose (Anser anser) (n=7) preen glands by using immunohistochemical methods. Cytoplasmic leptin immunostaining was detected in the sebaceous zone, in intermediate cells adjacent to germinative cells, in degenerative cells, and in secretion in the apical region of the glycogen zone. A strong leptin expression in the glycogen zone where an intense PAS and nonspecific esterase reactions are present may indicate that this hormone could act as a regulator for fatty acid homeostasis.

Key words: goose, leptin, preen gland.

The preen gland, also known as the oil gland or the uropygial gland, is a bilobar cutaneous gland in birds, which is located at the base of the tail and surrounded by a connective tissue capsule. It is especially bigger in water-birds than land-birds, and is present in most bird species while absent or vestigial in some other species (Rheidae, Psittacidae, Columbidae) (7, 20). Each lobe, which is separated by an interlobular septum, has a central cavity, which opens to the nipple-like papilla at the top of the skin via the central duct (2, 23). Preen gland secretion is of holocrine character and consists fatty acids and their esters, vitamin D precursors, extruded cells, and enzymes. The secretion, which is spread all over the plumage by the bird’s bill during preening, waterproofs the feathers, and makes them flexible. Furthermore, the bacteriostatic and fungistic properties of this secretion protects the bird’s skin against microorganisms (6, 9, 28).

Leptin is a 16 kDa polypeptide hormone. This cytokine-like hormone is mainly produced in the adipose tissue and released into the bloodstream when the fat storage increases (32). It was reported that leptin is produced in different mammals’ tissues, such as the stomach (3), placenta (17), skeletal muscles (31), mammary glands (29), pituitary gland (19), hypothalamus (26), salivary glands (16), and olfactory mucosa (4). Leptin not only regulates the food intake and energy expenditure, but also has a variety functions including reproduction (1), haematopoiesis (8), immunological reactions (24), angiogenesis (10), and osteogenesis (21).

Unlike the mammalian version, in the chicken leptin is expressed not only in the adipose tissue but also in the liver, which is a primary source of lipogenesis in avian species (30). This study aimed to investigate the expression and distribution of leptin in the goose preen glands and define its possible functions.

Material and Methods

Animals. The preen glands were taken from healthy male adult geese (Anser anser) (n=7) obtained from Kafkas University’s Animal Research Farm (Kars/Turkey). Tissue samples were collected in compliance with the approved Kafkas University Animal Care and Use Committee protocol.

Histochemical procedures. Some of the samples were fixed in 10% formalin for 24 h and then embedded in paraffin. Mallory’s modified triple staining (15) was used to demonstrate the general structure of the preen gland. To determine the histochemical structure of the gland, the other part of the samples was fixed in formol-calcium solution at 4°C. Finally, the samples were cut into 10-15 µm thick sections in a cryostat. Glycogen was demonstrated by periodic acid-Schiff (PAS) method (25) and the activity of nonspecific esterase by α-naphtyl acetate method (5).
**Immunohistochemical procedure.** For the immunohistochemistry, the streptavidin-biotin peroxidase complex (ABC) method was utilised for 5 µm thick sections. Endogenous peroxidase activity was blocked with a 0.3% hydrogen peroxide for 20 min, and then the slides were washed in 0.01 M phosphate buffered saline (PBS) solution, pH 7.4. The tissue sections were incubated with a polyclonal rabbit leptin antibody (Sigma, Saint Louis, USA) diluted to 1:100 in PBS. The slides were incubated with biotinylated goat anti-rabbit IgG (Dako Corporation, Carpinteria, USA) diluted in PBS (1:300) for 30 min, and peroxidase conjugated streptavidin (1:300) (Dako) for 30 min. 3,3’-diaminobenzidine tetrahydrochloride (0.5 mg/mL; Dako Corporation, Carpinteria, USA) for chromogen and haematoxylin for counterstaining were used. The sections were mounted with immunmount and examined under a light microscope (Olympus BX51, JAPAN). A rabbit serum without a primer antibody served as the negative control.

**Results**

**Histological and histochemical examinations.** The goose preen glands were enclosed by a capsule of connective tissue composed of many tubules, which were arranged radially and centrally from the peripheral zone and were ruptured in the central zone. The tubules’ epithelial cells are classified under four types: germinative cells, which lie on the basement membrane and have heterochromatic nuclei and flattened shape; intermediate cells with achromatic nuclei and which are polygonally shaped; secretory cells, which include lipid droplets in their cytoplasm and achromatic nuclei; and degenerative cells with pyknotic nuclei. The tubular lumens were wider in the central zone than in the peripheral zone because of thinner tubular walls due to the desquamation of degenerative cells (Fig. 1). In the PAS staining, positive reaction was seen in all cell layers of central tubules, and in the germinative cells and a few intermediate cells of the peripheral zone. On the basis of the PAS staining results, the peripheral tubules are described as the sebaceous zone, and the central tubules as the glycogen zone (Fig 2). All the tubular epithelial cells of the glycogen zone were strongly positive for nonspecific esterase activity, whereas the germinative cells, some intermediate cells, and the secretion of the sebaceous zone, were only positive for this enzyme. (Fig 3).

**Immunohistochemical examination.** The immunohistochemical localisation of leptin revealed regional differences in the goose preen glands. A negative leptin expression was detected in the basal region of the sebaceous zone, which is adjacent to the capsule (Fig. 4a), while towards the glycogen zone (the apical region of the sebaceous zone) a moderate to strong diffuse cytoplasmic reaction was detected in intermediate cells, especially in those adjacent to the germinative cells. A weak reaction was seen in degenerative cell membranes and secretion (Fig. 4b). A cytoplasmic reaction was observed in all the tubular epithelial cells except for the germinative cells. The cellular membranes reacted strongly to leptin antibody in the tubules of the basal region from the glycogen zone (adjacent to the sebaceous zone) (Fig. 4c). The degenerative cells and the secretion in the apical region of the glycogen zone where the central cavity opens into the central duct gave a strong positive reaction, but the reaction was very weak or negative in the other cells (Fig. 4d).

The distributions of glycogen, nonspecific esterase, and leptin in goose preen glands are shown in Tables 1 and 2.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Distribution of glycogen, nonspecific esterase and leptin in the sebaceous zone of the preen gland</th>
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<td>Basal region</td>
<td>Apical region</td>
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<tr>
<td>Germinative cells</td>
<td>Intermediate cells</td>
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<td>Leptin</td>
<td>-</td>
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<td>Glycogen</td>
<td>++</td>
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<td>Nonspecific esterase</td>
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Staining was scored as negative (-); negative or weak (-/+); weak (+); moderate (++); strong (+++).

<table>
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<th>Table 2</th>
<th>Distribution of glycogen, nonspecific esterase, and leptin in the glycogen zone of the preen gland</th>
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<td>Glycogen zone</td>
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<td>Basal region</td>
<td>Apical region</td>
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Staining was scored as negative (-); negative or weak (-/+); weak (+); moderate (++); strong (+++).
Fig. 1. Goose preen gland. PT - peripheral tubules; MT - central tubules. Mallory’s trichrome staining.

Fig. 2. Goose preen gland. S - sebaceous zone; G - glycogen zone. PAS.

Fig. 3. Goose preen gland. S - sebaceous zone; G - glycogen zone. α-naphtyl acetate method.

Fig. 4a. The basal part of the sebaceous zone. Absence of leptin immunostaining. Streptavidin-biotin peroxidase complex (ABC). b. Cytoplasmic leptin expression in the apical part of the sebaceous zone. ABC. c. The basal part of the glycogen zone. Diffuse leptin expression in the cytoplasm and the presence of a strong reaction in cellular membranes. ABC. d. The apical part of the glycogen zone. Strong leptin reaction in secretion. Negative or very weak reaction in tubule epithelium. ABC.

Discussion

In the goose preen glands, each lobe is made up of an outer zone (sebaceous zone) with high esterase activity, and an inner zone (glycogen zone), which are characterised by the presence of glycogen, acid phosphatase, and lipids soluble in osmic acid. In the present study, all the epithelial cells showed a strong PAS reaction in the inner zone, which is called the glycogen zone (12, 18), but nonspecific esterase activity was more prominent in the glycogen zone than in the sebaceous zone.

Leptin has been studied widely in mammals, but less in birds, since it was first described in 1994 (32). The leptin encoding gene was expressed in the liver and adipose tissue of the birds (30, 22). The chicken leptin gene encodes the 163 a.a. gene, and is expressed in both the adipose tissue and the liver, whereas the leptin expression is exclusively localised in the adipose tissue in mammals. The chicken leptin gene is in 83%, 96%, and 97% identical to the human, rat, and mouse gene, respectively (30). But there have been very few reports about the determination of the leptin distribution in birds using the immunohistochemical method. In a recent study, leptin-like protein distribution was reported in the chicken gastrointestinal tract (27).

In the present study, leptin-immunoreactive cells were demonstrated in both the sebaceous and glycogen zones of goose preen glands, with the intensities varying from zone to zone. A strong immunoreactivity was seen in the basal cells of the glycogen zone. These results are similar to the morphologic characteristics of the leptin immunoreactivity detected in the gastrointestinal tract of birds (27). The leptin immunoreactivity in the goose preen glands suggests both an exocrine and endocrine...
action for this hormone in birds similar to that in mammals (13, 14). Leptin is possibly secreted from the intermediate cells adjacent to the germinative cells in the apical regions of both the sebaceous and glycogen zones into the tubular lumen, possibly regulating some local activity. These findings are the first report of the presence of leptin in goose preen glands. The occurrence of leptin in the glands suggests that this peptide may influence several local activities, such as lipid handling. Multibranched fatty acids are synthesized in large quantities in the preen gland of geeze (11). Because of the strong leptin expression in the glycogen zone where strong PAS and nonspecific esterase reactions were detected, this may indicate that this hormone could act as a regulator for fatty acid homeostasis.

References