Turk J Vet Anim Sci
28 (2004) 403-407

Scientific Report

Immunohistochemical Distribution of Cells Containing Insulin, Glucagon and Somatostatin in the Goose (*Anser anser*) Pancreas

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Received: 05.02.2003

Abstract: The purpose of the present study was to determine the distribution of cells containing glucagon (A), insulin (B), and somatostatin (D) in the goose pancreas. Small pieces of tissue from 5 adult geese (*Anser anser*) pancreases were dissected under deep ether anaesthesia. Sections were stained with Crossmon's connective tissue stain for general observations and Gomori's method for pancreatic islet cells. Sections were further processed for standard immunohistochemical techniques using the avidin-biotin-complex method for the distribution of A, B and D cells. There were no B cells inside the A islets, which were mainly composed of A cells. B islets were mainly composed of insulin-producing B cells arranged in a circular fashion around blood capillaries. Granules containing insulin were localised in blood vessel sites of the B cells. Although D cells were distributed in almost all sites of the A islets, they were mainly localised at the periphery of the B islets. D cells throughout the pancreas were more common than that of B and A cells. The distribution of cells containing insulin, glucagon and somatostatin in the goose pancreas, with small differences, was similar to that of other avian species.

Key Words: Goose pancreas, immunohistochemistry, insulin, glucagon, somatostatin

İnsulin, Glukagon ve Somatostatin İçeren Hücrelerin Kaz (*Anser anser*) Pankreasında İmmunohistokimyasal Dağılımı

Özet: Bu çalışmanın amacı, insulin (B), glukagon (A) ve somatostatin (D) içeren hücrelerin kaz pankreasındaki dağılımını saptamaktı. Küçük doku parçaları anestezi altındaki beş erişkin kaz pankreasından alındı. Kesitler, genel gözlem için Crossman'ın bağ doku boyasıyla, pankreas adacıkları için ise Gomori'nin boyama metodu ile boyandı. A, B, ve D hücrelerinin dağılımı için, Avidin-Biotin-Kompleks metodu kullanıldı. A hücrelerinin çoğunlukta olduğu A adacıklarında B hücrelerine rastlanmadı. B adacıklarında hücrelerin çoğu kapillarların etrafında daire tarzında yerleşmiş B hücreleriydi, bu adacıklarda periferal yerleşimli A hücrelerine de rastlandı. İnsulin granülleri B hücrelerinin damar kutbuna yerleşmişti. D hücreleri A adacıklarının her tarafına dağılmış olmalarına rağmen, B adacıklarında genellikle periferal olarak yerleşmişti. Pankreas dokusunda genel olarak D hücreleri, A ve B hücrelerine göre daha yaygındı. Kazların pankreasında insulin, glukagon, ve somatostatin içeren hücrelerin dağılımının, küçük farklarla birlikte, diğer kanatlılarınki ile benzerlik gösterdiği saptanmıştır.

Anahtar Sözcükler: Kaz pankreası, immunohistokimya, insulin, glukagon, somatostatin.

Introduction

The avian pancreas is composed of 2 main lobes, dorsal and ventral, which extend from the apex of the duodenal loop to the point where the pancreatic ducts enter the distal duodenum. Another, smaller lobe, extending from the head of the pancreas towards the spleen (1), has been termed the splenic lobe. A further subdivision of the lobes of the pancreas has been made by Mikami and Ono (2), who divide the ventral lobe into the ventral lobe proper and the third lobe on the basis of the latter's independent form and peculiar distribution of islets. Functionally, the pancreas is divided into 2 types, the exocrine, enzyme-secreting cells and the endocrine, hormone-producing cells (3). The studies by Mikami and Ono (2) using conventional histological staining and by Rawdon and Andrew (4), using the immunoperoxidase method, showed that A, B, and D cells were regionally distributed and that the population of A cells was densest in the splenic lobe. Weir et al. (5) performed radioimmunoassay on extracts of various regions of the chicken pancreas and, in agreement with cell frequency measurements, found extremely high concentrations of

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glucagon and somatostatin in the splenic lobe. The purpose of the present study was to determine the distribution of A, B and D cells in the goose pancreas.

Materials and Methods

Tissue collection and staining

In the current study, 5 adult geese (*Anser anser*) were used. Small pieces of tissue were dissected under deep ether anaesthesia from the lobes of the pancreas. Samples were fixed in Bouin's fluid and then routinely processed for embedding in paraffin. Tissue blocks were cut on a microtome into 6 µm sections. Sections were stained with Crossmon's connective tissue stain (6) for general observations. Gomori's method (7) for pancreatic islet cells was used to determine the A and B islets.

Immunohistochemistry

The endogeneous peroxidase and non-specific binding sites for antibodies were suppressed by treating sections with 0.5% hydrogen peroxide for 30 min and 10% normal rabbit serum for 10 min at room temperature, respectively. Furthermore, sections were processed for standard immunohistochemical techniques using the avidin-biotin-complex (ABC) method (8). The working dilutions and the sources of antibodies used are listed in the Table. We used peptide specific antibodies isolated from mammalian species for a number of reasons. First, there was very high amino acid sequence homology between the mammalian-derived hormones used in our study and their bird counterparts. Second, previous studies demonstrated that mammalian-derived antibodies such as rabbit anti-somatostatin and rabbit anti-glucagon, as used in our investigation, cross reacted with their bird counterparts (4,9,10). Negative controls were carried out by incubating sections with phosphate-buffered saline (PBS) instead of the primary antiserum. Positive controls were also conducted with tissue sections from the gastrointestinal tract of rabbits known to contain the hormones studied. The sections were incubated in primary antisera in PBS-containing bovine serum albumin (2.5%) and Triton X-100 (0.2%) for 1 h at room temperature. Subsequently, the binding of primary antisera was detected using rabbit anti-mouse antisera and StreptABC. Finally, the chromogen protocol was used to reveal the distribution of bound peroxidase (11).

Table. The primary antibodies and their dilutions.

Antisera	Working Dilution	Sources
Somatostatin	1:100	Dako
Glucagon	1:40	Signet
Insulin	1:40	Signet
Goat anti-rabbit IgG	1:100	Zymed
Rabbit anti-mouse IgG	1:100	Dako
StreptABC	1:50	Dako

Results

The goose pancreas is located between the 2 arms of the duodenal loops, and also has dorsal, ventral, third and splenic lobes. The endocrine parts of the pancreas were scattered singly or in small groups of islets of various shapes and sizes in the intersititium of the exocrine part. The A islets, which were larger than the B islets, had no distinct borders with the exocrine part of the pancreas (Figure 1). The cells in the A islets were more densely populated than the B islets (Figure 1). There were no B cells inside the A islets, which were mainly composed of A cells (Figures 2 and 3), whereas there were some D cells in them (Figure 4). Although A islets were found in all the pancreatic lobes, they were mostly localised in the splenic and third lobes and less in the ventral lobe.

B islets, which were smaller than the A islets, were distinctly separated from the exocrine part of the pancreas with connective tissue layers. B islets were mainly composed of insulin-producing B cells arranged in a circular fashion around blood capillaries (Figure 3). Granules containing insulin were localised in the blood vessel site of the B cells. Cells containing insulin and glucagon, besides the B islets, were also scattered singly or in small groups in the intersititum of the exocrine part of the pancreas. There were also many D cells in the B islets (Figure 5), whereas very few A cells were observed at the periphery of those islets (Figure 2). As in the A islets mentioned above, even though B islets were observed in all the pancreatic lobes, they were mainly localised in the splenic and third lobes and less in the ventral lobe.

Although D cells were distributed in almost all sites of the A islets (Figure 4), they were mainly localised at the periphery of the B islets (Figure. 5). D cells throughout the pancreas were more common than that of B and A cells. In addition, D cells were found singly or in small

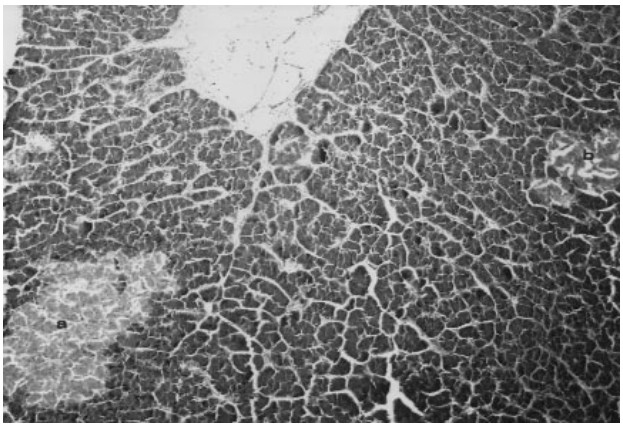

Figure 1. A islets (a) and B islets (b) in the goose pancreas. Gomori's staining method, x 20.

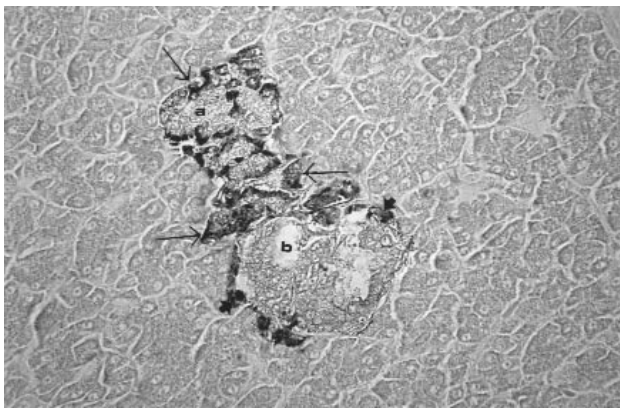

Figure 2. A islets (a) and B islets (b) in the goose pancreas and glucagons-containing cells (A cells) in A islets (thin arrows), and in B islets (thick arrows), x 40.

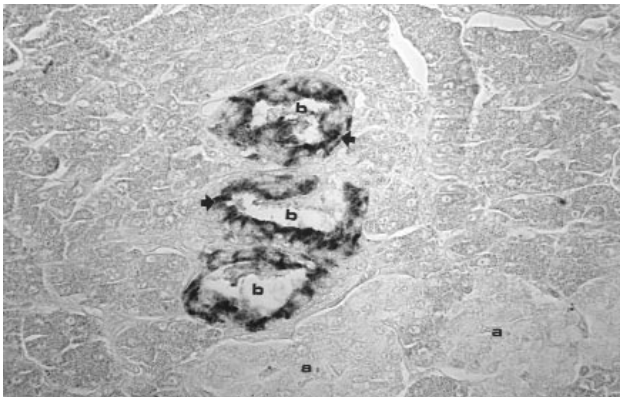

Figure 3. Arrows indicate cells containing insulin (B cells) in B islets (b). A islets (a), x 40.

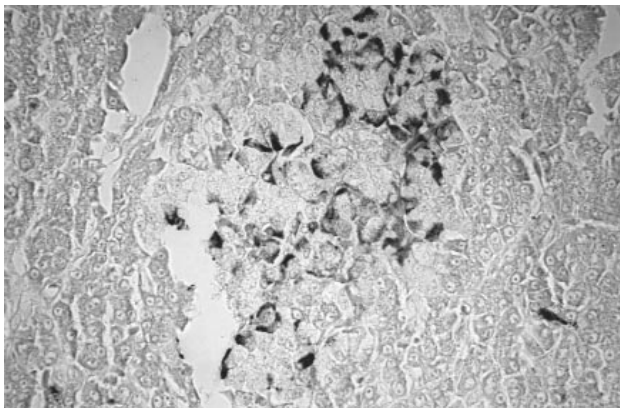

Figure 4. Cells containing somatostatin (D cells) were scattered throughout the A islets, x 40.

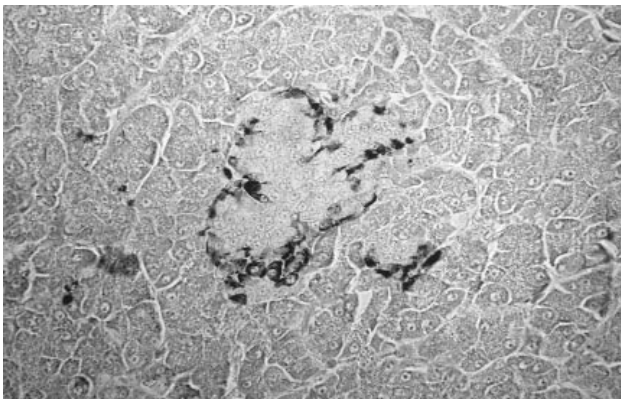

Figure 5. Cells containing somatostatin (D cells) were mainly localized at the periphery of the B islets, x 40.

groups in the exocrine part of the pancreas. Granules containing somatostatin were scattered throughout the cytoplasm of those cells (Figures 4 and 5). Cells containing somatostatin in the pancreas were more common than those containing insulin and glucagon.

Discussion

The goose pancreas, as in other avian species (1,12), is located between the 2 arms of the duodenal loops and also has dorsal, ventral, third and splenic lobes. Based on the argyrophil reaction of the cells, the avian pancreas is divided into "dark", "light" and "mixed" islets. However,

the exact border between the endocrine and exocrine parts of the pancreas is not always distinguished (13).

Glucagon-producing cells were mainly found in A islets (14-16). B islets were separated from the exocrine part of the pancreas with a thin layer of collagen. Approximately 80% of the B islets were made up of B cells found in small groups at the centre of those islets. In addition, a few B cells were found in the A islets (14). However, in the current study, we did not find any B cells in the A islets, and B cells were located at the periphery of the B islets. Thus, with the exception of these 2 findings, our results on the goose pancreas were in agreement with those of previous studies.

Somatostatin-producing D cells were seen in both A and B islets (2,14,17,18). Although those cells were scattered throughout the A islets, they were mainly localised at the periphery of the B islets. Furthermore, A, B and D cells, in addition to those islets, were also found singly or in small groups in the exocrine part of the pancreas (4,14).

Tomita et al. (19) demonstrated that the highest levels of insulin, glucagon and somatostatin concentrations were found in the splenic lobe. In addition, Oakberg (20) showed that B islets were mainly found in the splenic lobe, whereas other researchers (2,13,15,21) demonstrated that A islets were predominantly found in both the splenic and third lobes. Our findings are in agreement with those previous results, as shown in Figures 2-4. Mixed islets as shown in the chicken, which were structurally similar to mammalian species (16), were not noticed in the goose pancreas. In conclusion, the distribution of cells containing insulin, glucagon and somatostatin in the goose pancreas, was with small differences, similar to that of other avian species.

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