Journal of Animal and Veterinary Advances 6 (5): 693-696, 2007

Immunohistochemical Study on the Endocrine Cells in the Pancreas of the Ostrich (*Struthio camelus)*

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Abstract: Insulin, glucagon and somatostatin immunoreactivity were found in ostrich pancreatic endocrine islet cells. Insulin immunoreactive cells were present in the central regions with high frequency. Glucagon immunoreactive cells were occurred in the peripheral regions with moderate frequency and a few of these cells were also demonstrated in the mantle regions. Somatostatin immunoreactive cells were detected in the mantle, peripheral and central regions with moderate frequency. In the exocrine pancreas, insulin, glucagon and somatostatin immunoreactivity were not detected. Gonadotropin Releasing Hormone (GnRH) immunoreactivity was not observed in the pancreatic endocrine and exocrine regions.

Key words: Insulin, glucagon, somatostatin, GnRH, ostrich, pancreas

INRODUCTION

It is generally known that pancreas of vertebrates is subdivided into two regions. One is an exocrine region where digestive enzymes are released and the other is an endocrine portion where regulatory hormones such as insulin, glucagons and somatostatin are released into the blood vessels (Ross *et al*., 1995).

Generally, the avian pancreas consisted of 2 lobes; dorsal and ventral (Bailey *et al*., 1997). But many reports generally indicated that the pancreatic lobes of avian species consisted of four lobes: Splenic, third, dorsal and ventral (Clara, 1924; Mikami and Ono, 1962; Hodges, 1947). Many researches have shown concern for the anatomical, histological and endocrinological structure of the pancreatic lobes. Existence of various hormone producing cells was demonstrated in the pancreas of avian species including chicken (Kalliecharan and Steeves, 1982; Cooper *et al*., 1997) duck (Lucini *et al*., 1996) and mallard (Lee *et al*., 1998a, b) using immunohistochemistry. However, no reports show distribution of immunoreactive endocrine cells in the pancreas of ostrich (*Struthio camelus*). Ostrich belong to the *Struthionidae* family and are largest living bird in the world (Kumari and Kemp, 1998).

In the present study, the distribution and relative frequency of endocrine cells in ostrich *(Struthio camelus*) pancreas was examined by immunohistochemical method using specific antisera against insulin, glucagon, somatostatin and Gonadotropin Releasing Hormone (GnRH). This study also aimed at ascertaining whether their distribution is similar to those of avian species.

MATERIALS AND METHODS

Animals and tissue samples: Six adult ostriches male were used. Birds with body mass of 45-60 kg were anaesthetized by injecting pentobarbitone sodium. The left carotid artery was cannulated at the base of the neck and allowed to exsanguinate. Tissue samples were taken from oesaphagus and fixed in 4% neutral-buffered formalin for 24 h. They were then dehydrated through graded ethanol and embedded in paraffin. Seven μm-thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemistry: Immunohistochemical staining was carried out by the peroxidase linked Avidin-Biotin Complex [ABC] method and Peroxidase-Anti Peroxidase [PAP] method. Blocking of endogenous peroxidase activity was done with 0.08% Hydrogen peroxide (H_2O_2) in methanol for 5 min (Sternberger, 1986). In order to block unspecific binding incubation with (1:10) normal goat serum in 0.1 M PBS, pH 7.2 was performed.

The ABC method: Sections were incubated for 16-20 h at 4° in mouse anti-insulin IgG (Chemicon, MAB 1124). The antibodies were diluted to 1:500 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin. Sections were then incubated in biotinylated goat anti-mouse IgG

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(Sigma, B9904) and followed with streptavidin horseradish peroxidase (Chemicon, 20774) both at a dilution of 1:50 in PBS, for 1 h at room temperature. Sections were washed in PBS for 30 minutes after each incubation. Sections were then immersed in glucose oxidase diamino benzidine nickel ammonium sulphate (GDN) substrate (Shu *et al*., 1988) for 10 min, washed in distilled water and counterstained with eosine.

PAP (Peroxidase-Anti-Peroxidase) method: Sections were incubated for 16-20 h at 4°C with rabbit IgG antibodies against glucagon (Chemicon AB932), somatostatin-14 (Chemicon, AB1976) and gonadotropin releasing hormone (Chemicon, AB1567). Antibodies were diluted to 1:500, 1:500 and 1:100 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin respectively. Sections were then incubated in goat anti-rabbit IgG (Dako, Z0421) followed by rabbit peroxidase anti-peroxidase complex (Zymed Lab., 61.2003) both at dilution of 1:50 in PBS, for 1 h at room temperature. Sections were washed in PBS for 30 min after each incubation and finally immersed in glucose oxidise-DAB-nickel ammonium sulphate substrate (Shu *et al*., 1988) for 10 minutes. After washing in distilled water and counterstaining with eosin, sections were dehydrated and coverslips mounted with aqueous permanent mounting medium.

The specificity of each immunohistochemical reaction was determined as recommended by STERNBERGER (Sternberger, 1979) by using (including the replacement of) specific antiserum preincubated with its corresponding antigen. Sections were examined with light microscope and photographs were taken.

RESULTS

In this study, all three kinds of the immunoreactive endocrine cells were detected using antisera against insulin, glucagons and somatostatin in the pancreatic islets which where distinguished as three distinct layers, a central region, mantle zone and peripheral region.

Table 1: Regional distribution and relative frequencies of the insulin, glucagon, somatostatin and Gonadotropin releasing hormone (GnRH) immunoreactive cells in the pancreatic endocrine and exocrine regions of the ostrich (*Struthio camelus)*

Immunoreactive cells	Pancreatic endocrine region			Pancreatic exocrine region
	Central	Mantle	Peripheral	
Insulin	+++	-	-	-
Glucagon	-	+	++	-
Somatostatin	++	++	++	-
GnRH	-	-	-	-

Relative frequencies; +++: Numerous, ++: Moderate, +: Rare, -: Not derected

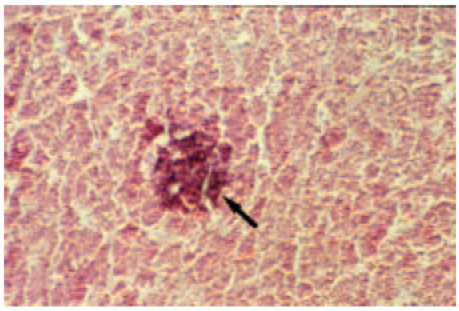

Fig. 1: Insulin immunoreactive cells in the pancreatic islet of ostrich (arrow). Note that most of the immunoreactive cells were located in the central regions of pancreatic islets. X 200

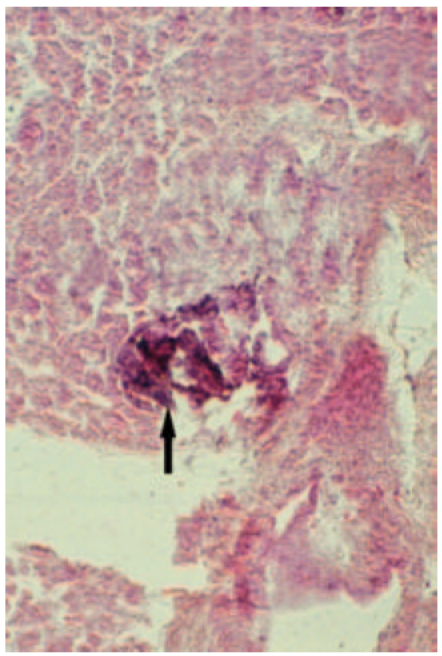

Fig. 2: Glucagon immunoreactive cells in the pancreatic islet of ostrich (arrow). Note that most of the immunoreactive cells were located in the peripheral regions of pancreatic islets. X 200

Different regional distribution and relative frequencies of these immunoreactive cells were observed in the different pancreatic regions and these differences are shown in Table 1. Spherical to spindle or oval to round-shaped immunoreactive cells were observed in pancreatic islets of the ostrich.

Insulin immunoreactive cells were located towards the centre of the pancreatic islet with numerous frequencies (Fig. 1). Glucagon immunoreactive cells were

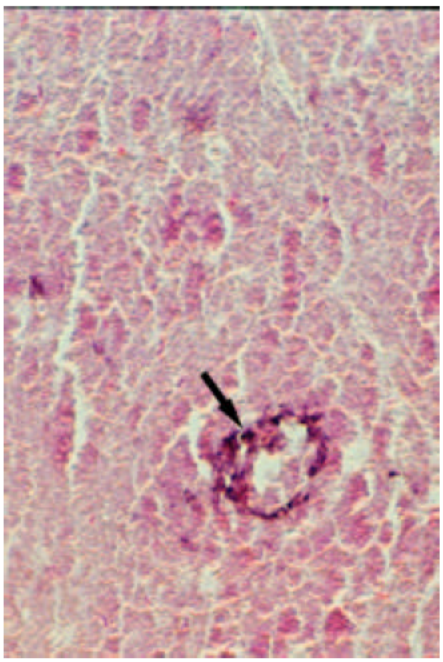

Fig. 3: Somatostatin immunoreactive cells in the pancreatic islet of ostrich (arrow). Note that some of the immunoreactive cells were located in the mantle regions of pancreatic islets. X 200

observed in the mantle and peripheral regions of the pancreatic islets with rare and numerous frequencies respectively (Fig. 2). Somatostatin immunoreactive cells were found in the mantle, peripheral and cenral regions of pancreatic islet with moderate frequencies (Fig. 3).

In addition to pancreatic islet regions, pancreatic exocrine region was also examined using these antisera and also antisera against GnRH. No positive staining was observed for insulin, glucagon, somatostatin and GnRH in exocrine region of ostrich pancreas. GnRH immunoreactivty was also not detected in the pancreatic islet cells.

DISCUSSION

Insulin is a polypeptide hormone that regulates carbohydrate metabolism. Apart from being the primary agent in carbohydrate homeostasis, it has effects on fat metabolism and it changes the liver's activity in storing or releasing glucose and in processing blood lipids and in other tissues such as fat and muscle. Insulin is synthesized in the B cells of the pancreatic islets (Hsu and Crump, 1989). The distribution and relative frequency of the insulin-immunoreactive cells in the pancreas have been reported in the chicken (Bagnell *et al*., 1989; Ku *et al*., 2000), birds (Mensoh Brown *et al*., 2000) goose (Gulmez *et al*., 2004) and duck (Tomita *et al*., 1985). From the those reports it is known that insulin immunoreactive cells are situated in the central regions of the avian pancreas and that other cells such as glucagon and somatostatin immunoreactive cells surround them. In the present study, like the other avian species, insulin immunoreactive cells were demonstrated in the centre of the pancreatic islets.

Glucagon is synthesized in A cells of the pancreas and regulated glucose levels in blood (Hsu and Crump, 1989). In the present study, glucagon-immunoreactive cells were mainly found in the peripheral region of ostrich pancreatic islets. Glucagon-immunoreactive cells of the avian pancreas have been reported to be occurred in peripheral region (Lucini *et al*., 1996; Mensha Brown *et al*., 2005; Gulmez *et al*., 2004; Tomito *et al*., 1985). Our results on the ostrich pancreas were in agreement with those of previous studies.

The straight and cyclic forms of somatostatin, consisting of 14 amino acids were isolated from the hypothalamus of the sheep for the first time (Brazeau *et al*., 1973). Somatostatin-immunoreactive cells were detected in both A and B islets in the endocrine pancreas and in small groups in the exocrine pancreas of some avian species (Lee *et al*., 1996; Kue *et al*., 2000; Gulmez *et al*., 2004). In the present study, similar to those of other avian species (Lucini *et al*., 1996; Mensha Brown *et al*., 2005; Gulmez *et al*., 2004; Tomito *et al*., 1985) somatostatin immunoreactive cells were mainly distributed in the peripheral and central region of pancreatic islets. In the present study, somatostatin immunoreactivity was also observed in the mantle region of ostrich pancreatic islets.

Recently, details about the functional roles of GnRH in various extrapituitary tissues are continuously being discovered. It was reported by Wang *et al*. (2001) and our recent research (Tarakcy *et al*., 2005, 2007) that GnRH is expressed in normal pancreatic tissue and especially in the exocrine part of some mammals. Contrary to above results, in the present study, GnRH immunoreactivity was not demonstrated in exocrine and endocrine part of ostrich pancreas. The non-existence of GnRH in ostrich exocrine pancreas suggests that there is some differentiation between enzyme/hormone product cells in exocrine pancreas of avian and mammalian species. Future studies requires in exocrine pancreas of other avian species with regard to GnRH immunoreactivity, to support this hypothesis.

CONCLUSION

The regional distribution of endocrine cells in the pancreatic islets of ostrich *(Struthio camelus)* was found

to be similar to that of other avian species. But the non-existence of GnRH immunoreactive cells in exocrine pancreas was quite different from that of mammals.

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