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An immunohistochemical study of the endocrine pancreas in raptors

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ABSTRACT

The cytoarchitecture of the endocrine pancreas of 10 raptors (golden eagles, peregrine falcons, Saker falcon, turkey vultures, red-tailed hawk and unspecified falcon) was examined by immunohistochemistry. Three islet types were identified: type A mixed islets composed mainly by glucagon (A)-secreting cells, type B mixed islets with predominantly insulin (B)-secreting cell component and type M mixed islets (type M) consisting of variable number of glucagon-, insulin- and somatostatin (D)-secreting cells. The latter were further characterized into Type I, II or III according to the cell distribution of the three cell types. A and D cells were also randomly scattered within the exocrine pancreas. The results of this study suggest that the classical concept in birds of a segregation of A and B cells in well-defined and distinct islets is not applicable in raptors, reflecting an evolutionary adaptation to different dietary habits and variation in developmental mechanisms.

1. Introduction

A great diversity of islet structure exists among vertebrates, suggesting multiple mechanisms of islet formation and variation in developmental processes, although different metabolic requirements and physiological conditions may play a more prominent role in determining islet structure (Steiner et al., 2010). The endocrine portion of the avian pancreas occupies more tissue mass than in mammals and the distribution of cell types differs as well. Three types of islets have been classically described; dark islets composed mostly of glucagon-secreting A cells with some D cells producing somatostatin, light islets containing a mixture of D and insulin-producing B cells, and mixed islets with all three cell types (Pilny, 2008). Clara (1924) and Nagleschmidt (1939) have indicated that the bird pancreas contains proportionately more A cells than does the mammalian pancreas, thus confirming the important role played by glucagon in maintaining the blood glucose balance in birds. However, some physiological differences have been observed among different avian species. For example, glucagon does not affect plasma insulin concentration in fowl, whereas it markedly increases this concentration in ducks (as well as in man and dogs) (Langslow and Hales, 1971). Moreover, Algauhari and Amer (1966) have noticed striking differences in the proportional population of A and B cell types in the pancreatic islets of granivorous and carnivorous birds. Seasonal changes in the islet tissue have been observed in the European blackbird (*Turdus merula*) (Epple, 1961) and the starling (*Sturnus vulgaris*) (George and Naik, 1964). In pigeon, though A and B cell types undergo cyclic changes in the secretory activity, there is no significant alteration in the numerical population of these cells types (Guha, 1977). In some birds, such as the zebra finch (*Taeniopygia guttata*), B cells are rare and D cells are more common, but the effect of this difference on the islets function is unknown (Kim et al., 2010).

Studies on the distribution of hormonal peptides in the endocrine pancreas have been performed on domestic fowl (Do Prado et al., 1989; Falkmer, 1985; Iwanaga et al., 1983; Mikami and Ono, 1962; Rawdon, 1998; Schwarz et al., 1983; Tomita et al., 1985; Watanabe and Nagatsu, 1991; Watanabe et al., 1988, 1990), duck (Falck and Hellman, 1963; Lucini et al., 1996), swan (Schwarz et al., 1983), pigeon (Roth, 1968), sparrowhawk (Kara et al., 2014), Japanese quail (Mikami et al., 1985), Brazilian sparrow (Nascimento et al., 2007), Houbara bustard (Mensah-Brown et al., 2000), goose (Gulmez et al., 2004), parakeet (Gupta and Kumar, 1980) and buzzard (Bayrakdar et al., 2011).

To the authors' knowledge, only two publications on the endocrine pancreas in raptors are available (Edwin and Leigh, 1993; Simsek et al., 2008). However they are mostly incomplete since the islet structure is described only in one species each (eagle the former, falcon the latter) and the immunohistochemical characterization performed by Simsek et al. (2008) does not include somatostatin and glucagon.

Therefore, the aim of this study is to characterize the immunohistochemical features of the endocrine pancreas of different raptors

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and to ascertain whether the distribution of endocrine cells is similar to those of other avian species and mammals.

2. Materials and methods

2.1. Samples

Ten formalin-fixed paraffin-embedded samples from the database of the California Animal Health and Food Safety Laboratory System (CAHFS, Fresno, CA, USA) were included in this study. The samples consisted of pancreatic tissue collected from raptors and specifically 3 golden eagles (*Aquila chrysaetos*), 2 peregrine falcons (*Falco peregrinus*), 1 unspecified falcon, 1 Saker falcon (*Falco cherrug*), 2 turkey vultures (*Cathartes aura*) and 1 red-tailed hawk (*Buteo jamaicensis*). None of the birds had any evidence of pancreatic damage such as vacuolations, degeneration, necrosis or inflammation. Three birds were euthanized because of multiple fractures, three other birds had respiratory aspergillosis, one bird was affected by respiratory cryptosporidiosis and rest of the birds had miscellaneous conditions of unknown significance. The distribution of endocrine cells was compared with that reported in other species (Heller, 2010; Steiner et al., 2010). Specimens were representative of the three lobes of the pancreas: the dorsal, ventral, and splenic lobes. An overall histological evaluation of the exocrine and endocrine pancreas was performed on hematoxylin & eosin-stained slides.

2.2. Immunohistochemical staining

For immunohistochemistry (IHC), tissue sections were dewaxed in xylene and rehydrated in an alcohol series. After inhibition of the endogenous peroxidase activity and blocking with 10% normal goat serum, the slides were incubated for 60 minutes with the following primary antibodies: polyclonal guinea pig anti-insulin (1:600; Dako), polyclonal guinea pig anti-glucagon (1:100; LINCO Research) and polyclonal rabbit anti-somatostatin (1:1000; ImmunoStar). The incubation with the secondary biotinylated goat anti-guinea pig IgG (1:250) was followed by the streptavidin–biotin–peroxidase complex. The presence of antibody binding was 'visualized' with aminoethylcarbazole and sections were counterstained with Mayer's hematoxylin. Positive control slides were used for each antibody using pancreas of dogs and different avian species (turkey, chicken, duck, psittacines). A negative control was performed in all instances by omitting the primary antibody and incubating tissue sections with Tris buffered saline (TBS).

3. Results

All three types of endocrine cells were detected using antisera against insulin, glucagon and somatostatin in the pancreatic islets. Lack of cross-reactivity of the anti-somatostatin antibody was observed in the three golden eagles. Most of the positive cells were organized into islets, although they were also seen throughout the exocrine parenchyma. No segregation into pure A and B islets was observed but all the endocrine cell types were represented in each islet, although with variable number and distribution. Therefore three islet types were identified in the pancreas of raptors: type A mixed islets composed mainly by glucagon (A)-secreting cells (Fig. 1), type B mixed islets with predominantly insulin (B)-secreting cell component (Fig. 2) and pure mixed islets (type M) consisting of variable number of glucagon-, insulin- and somatostatin (D)-secreting cells (Fig. 3). The latter were the most prevalent in the endocrine pancreas of 1/3 golden eagle and 2/2 turkey vultures. The three types of islets were equally represented in the other raptors. Overall, insulin-immunoreactive cells (B cells) in type M islets were more numerous compared with A and D cells.

The islets were distributed throughout the lobes without any predilection in 1/3 golden eagle, 1/1 unspecified falcon and 2/2 turkey vultures, while in 2/3 golden eagles, in 2/2 peregrine falcons and 1/1 Saker falcon the splenic lobes contained the highest number of types A and B islets.

A cells (glucagon-positive) were mainly distributed in the type A islets and in the M islets. Few scattered A cells were also observed at the periphery of the type B islets and within the exocrine pancreas. Occasionally, A cells showed cytoplasmic processes.

B cells (insulin-positive) were mainly observed in type B islets with occasional circular arrangement around a central small capillary (1/3 golden eagles; 2/2 peregrine falcons; 2/2 turkey vultures; 1/1 red-tail hawk) or as a round central aggregate (1/1 Saker falcon; 1/2 unspecified falcons; 2/2 turkey vultures; 1/1 red-tail hawk), as well as in M islets. Few isolated B cells were distributed at the periphery of the type A islets or scattered within the exocrine tissue.

Somatostatin-positive D cells were observed in A, B and M islets, although more numerous in the former (2/2 peregrine falcons) and latter ones (1/1 Saker falcon; 1/1 unspecified falcon; 2/2 turkey

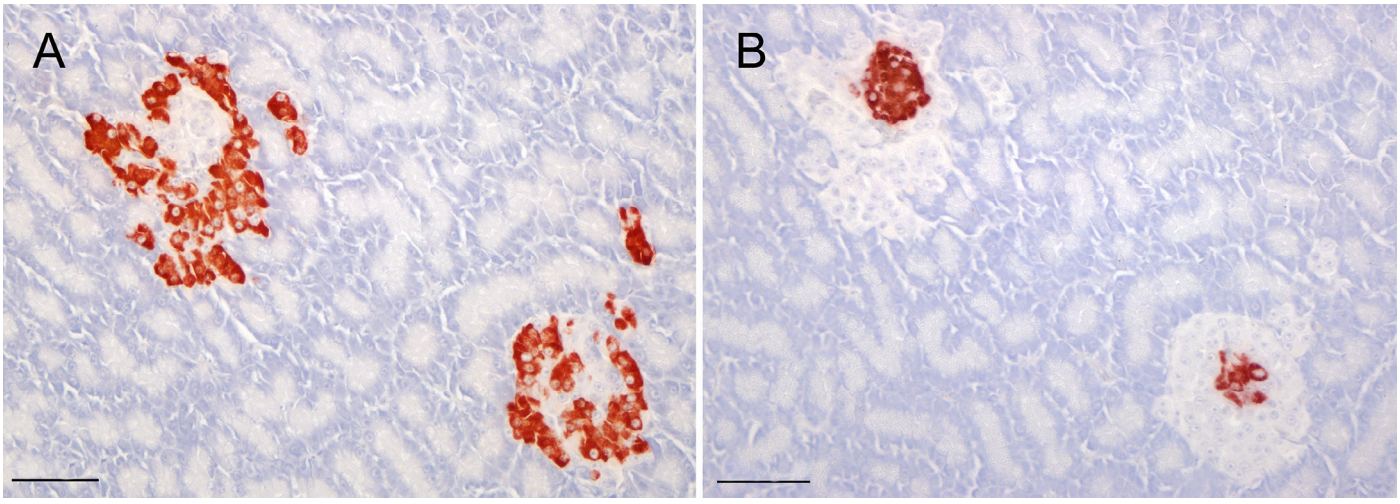

Fig. 1. Type A mixed islets in the endocrine pancreas of a turkey vulture. Numerous glucagon-positive A cells (A) in the outer layer encircling a small aggregate of insulin-positive B cells (B). Immunohistochemistry (IHC). Aminoethylcarbazole and Mayer's hematoxylin counterstain. Bar = 50 μm.