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≪Research Note≫

Glucagon-like Peptide-1 Receptor Expression in the Pancreatic D Cells of Three Avian Species; White Leghorn Chickens, Northern Bobwhites, and Common Ostriches

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Glucagon-like peptide (GLP)-1 is released from the intestinal L cells in response to food ingestion and stimulates insulin secretion from the pancreatic B cells, by binding to its specific receptor (GLP-1R), which is expressed on the pancreatic B cells in the mammalian pancreas. Previously, we demonstrated that chicken GLP-1R was expressed on the pancreatic D cells by using a specific antibody against chicken GLP-1R. In the present study, we compared the localization of GLP-1R in the pancreases of three avian species; white leghorn chicken, northern bobwhite, and common ostrich, using the double immunofluorescence technique. We found that the types of pancreatic islets in the northern bobwhite pancreas were similar to those found in the chicken pancreas. The ostrich pancreas contained several types of pancreatic islets. GLP-1R-immunoreactive cells were found in all types of pancreatic islets in both northern bobwhite and ostrich and expressed somatostatin immunoreactivity. The present results indicate that the pancreatic D cells are the target cells of GLP-1, and GLP-1 might play a physiological role via somatostatin in the avian species.

Key words: common ostrich, glucagon-like peptide-1 receptor, immunohistochemistry, northern bobwhite, pancreatic islet, white leghorn chicken

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Introduction

Glucagon-like peptide (GLP)-1 is an incretin hormone released from the endocrine cells scattered throughout the intestinal epithelium. It stimulates insulin secretion from the pancreatic islets in mammalian species. The mRNA of proglucagon, which acts as the precursor of GLP-1, is highly expressed in the ileum of the chicken alimentary tract (Honda *et al*., 2017). In chickens, GLP-1 is stored in the secretory granules of L cells, which are mainly distributed in the distal ileum (Hiramatsu *et al*., 2003, 2005; Nishimura *et al*., 2013) and is secreted in response to dietary protein and amino acids (Monir *et al*., 2014a; Nishimura *et al*., 2015). GLP-1 secreted from L cells binds to its specific receptor, GLP-1R. Mammalian GLP-1R comprises 463 amino acids and belongs to a family of G protein-coupled receptors with seven transmembrane domains (Thorens, 1992; Thorens *et al*., 1993). This receptor is widely expressed in many organs, including pancreas, heart, lung, and kidney. B cells, the main target cells of GLP-1 express GLP-1R in the mammalian pancreas. Fehmann and Habener (1991) have demonstrated that pancreatic B cells are directly influenced by GLP-1(7−37) by its binding to GLP-1R. Pyke *et al*. (2014) have revealed that GLP-1R is predominantly localized on B cells in monkey and human pancreases. Hence, GLP-1R is an important target for diabetes therapy (Teitelman, 2014).

Richards and McMurtry (2008) have demonstrated that GLP-1R gene is highly expressed in the gastrointestinal tract, brain, pancreas, and abdominal fat of chicken. Huang *et al*. (2012) have cloned the full-length cDNA of GLP-1R from chicken brain tissue and have demonstrated the expression of GLP-1R mRNA in some organs, including pancreas. Our previous study using a specific antibody against chicken GLP-1R (cGLP-1R) indicated that cGLP-1R was expressed on the pancreatic D cells and not on B cells (Watanabe *et al*., 2014). However, it is not clear whether this difference in

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GLP-1R expression observed in the mammalian pancreatic islets is a characteristic of avian species. The present study aimed to compare the expression of GLP-1R on the pancreatic islets between three avian species; white leghorn chicken (*Gallus gallus domesticus*), northern bobwhite (*Colinus virginianus*), and common ostrich (*Struthio camelus*), using the double immunofluorescence method, and to determine whether there is a difference in the GLP-1R expression among the islet types.

Materials and Methods

Experimental Birds

Young adult female ostriches ($n=3$, before laying period), weighing approximately 120 kg on an average, were obtained from a local farm. Adult male northern bobwhites ($n=6$), weighing approximately 200 g on an average, were obtained from the Avian Bioscience Research Center, Nagoya University Graduate School of Bioagricultural Sciences. Adult white leghorn chickens of both sexes ($n=6$, four males and two females), weighing approximately 1.8 kg on an average, were obtained from a local farm, kept in our laboratory under controlled light condition (12:12 h light:dark), and fed on a commercial diet and tap water *ad libitum*. The chickens and northern bobwhites were sacrificed by decapitation under anesthesia with sodium pentobarbital. The ostriches were sacrificed by bleeding from the cervical artery after electric shock. The pancreases of all the birds were rapidly dissected out as tissue samples. All procedures and animal treatments performed in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Shinshu University.

Tissue Preparations

The pancreases from all the birds were cut into small pieces with a razor blade and immersed in Bouin's fluid for 24 h at room temperature after several washings with 0.75% sodium chloride solution. The fixed tissues were embedded in paraffin wax according to the standard procedures, sliced into 5 μm paraffin sections, and mounted on silane-coated glass slides (Matsunami, Tokyo, Japan).

Immunohistochemistry

The double immunofluorescence method for GLP-1R and islet hormones was performed as described previously (Nishimura *et al*., 2017). Rabbit anti-chicken GLP-1R serum (1:500, Watanabe *et al*., 2014) was used for detecting GLP-1R localization. Guinea pig anti-glucagon serum (1:200, T-5037, Peninsula Laboratories, San Carlos, CA, USA), guinea pig anti-insulin serum (1:800, 5330-0054, Biogenesis, Poole, Dorset, UK), and rat anti-somatostatin monoclonal antibody (1:500, MAB354, Chemicon, Temecula, CA, USA) were used for detecting pancreatic A, B, and D cells, respectively. A cocktail of DyLight 549-labeled donkey antiserum against rabbit IgG (1:300; 611−700−127, Rockland Immunochemicals, Gillbertsville, PA, USA) and DyLight 488-labeled goat antiserum against guinea pig IgG (1:300, 606−141−129, Rockland Immunochemicals, Gillbertsville, PA, USA) or rat IgG (1:300; 610−741−124, Rockland Immunochemicals, Gillbertsville, PA, USA) was used as the secondary antibody. Sections were covered with a coverslip using an aqueous mounting medium (PermaFluor, Thermo Fisher Scientific, Fremont, CA, USA), observed and photographed under a fluorescence microscope (AxioImagerA1, Zeiss, Göttingen, Germany).

Results

Cells showing GLP-1R immunoreactivity were observed in the pancreases of three avian species evaluated in this study. These cells were located in the pancreatic islets; however, they were not observed in single cells scattered throughout the exocrine tissue. GLP-1R-immunoreactive cells in the pancreatic islets of the chicken pancreas also showed somatostatin immunoreactivity (Figs. 1a−c); however, no insulin or glucagon immunoreactivity was observed (Figs. 2a, d). Somatostatin-immunoreactive cells in mixed type of islet also showed GLP-1R immunoreactivity. Pancreatic islets in the northern bobwhite pancreas contained GLP-1R-immunoreactive cells, which also exhibited somatostatin immunoreactivity (Figs. 1d−f); however, no insulin or glucagon immunoreactivity was observed (Figs. 2b, e). Pancreatic islets in the ostrich pancreas contained many somatostatin-immunoreactive cells, and these cells in the pancreatic islets also demonstrated GLP-1R immunoreactivity (Figs. 1g−i). Cells showing insulin and glucagon immunoreactivity were immunonegative for GLP-1R (Figs. 2c, f). GLP-1R-immunoreactive cells were observed in all types of pancreatic islets of the pancreases of chicken, northern bobwhite, and ostrich, and also demonstrated somatostatin immunoreactivity.

Discussion

We demonstrated GLP-1R localization in the pancreases of the three taxonomically different avian species; white leghorn chicken, northern bobwhite, and common ostrich. White leghorn chicken (*G. gallus domesticus*) is a domesticated fowl belonging to the family Phasianidae. Northern bobwhite (*C. virginianus*) is a New World quail belonging to the family Odontophoridae, whereas Japanese quail (*Coturnix japonica*) is an Old World quail belonging to the family Phasianidae. Common ostrich (*S. camelus*) is a ratite and belongs to the family Struthionidae.

GLP-1 is an intestinal hormone released from the L cells in response to food ingestion. L cells are mainly distributed in the distal ileum of the chicken alimentary tract (Hiramatsu *et al*., 2003, 2005) and secrete GLP-1 in response to ingestion of foods (Monir *et al*., 2014b), particularly those containing dietary protein (Monir *et al*., 2014a) and amino acids (Nishimura *et al*., 2015). GLP-1 released from the intestinal L cells binds to its specific receptor, GLP-1R. Our previous study using a specific antiserum against chicken GLP-1R revealed that GLP-1R was expressed on the cells demonstrating somatostatin immunoreactivity, namely D cells, in the chicken pancreatic islets (Watanabe *et al*., 2014). The present study also showed the presence of GLP-1R-immunoreactive cells in the pancreatic islets of northern bobwhites, ostriches, and chickens using the same antiserum as the first

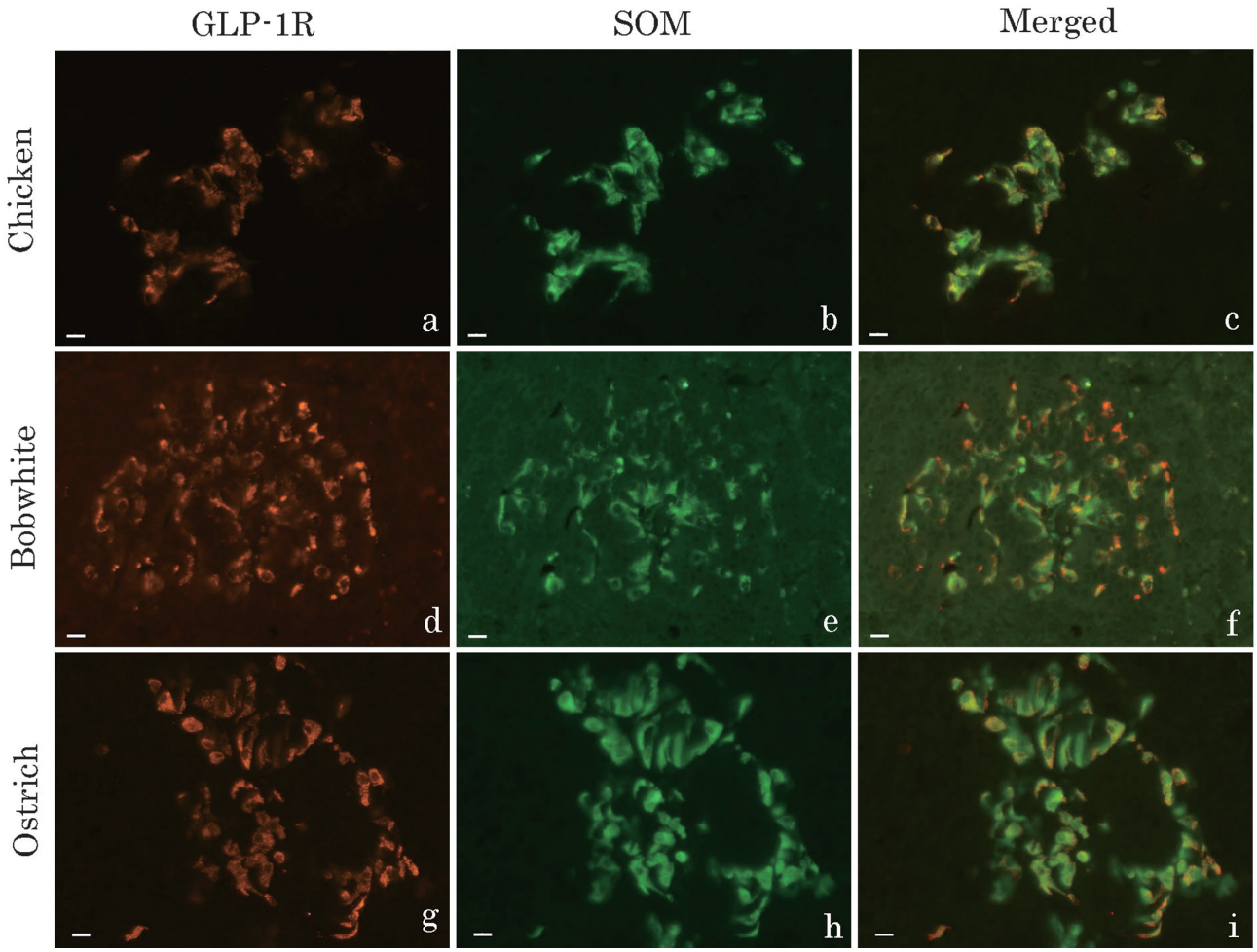

Fig. 1. **Double immunofluorescence images of glucagon-like peptide-1 receptor (GLP-1R, a, d, g) and somatostatin (SOM, b, e, h) in the pancreatic islets of chickens (a–c), northern bobwhites (d–f), and ostriches (g–i).** Figures **c**, **f**, and **i** show merged images of a and **b**, **d** and **e**, and **g** and **h**, respectively. Almost every SOM-immunoreactive cell in the pancreatic islets of three avian species also demonstrated GLP-1R immunoreactivity. Bars indicate 10 μm.

antibody. This result suggests that these three avian species exhibit high homology in the GLP-1R amino acid sequence and the pancreatic islet is a target tissue for GLP-1 in the avian species similar to that in the mammalian species. The amino acid sequence of GLP-1R shows relatively high homology among mammals. For example, the amino acid sequence of the rat GLP-1R shows 95% homology with the human form (Thorens, 1992; Thorens *et al*., 1993). Huang *et al*. (2012) characterized cGLP-1R and reported that cGLP-1R comprised 459 amino acids and shared higher amino acid sequence identity with the GLP-1R of human (79%) and rat (80%). It is assumed that northern bobwhite and ostrich GLP-1Rs also exhibit relatively high homology in the amino acid sequence with that of mammalian GLP-1Rs.

Several studies have demonstrated GLP-1R localization in the mammalian pancreatic islets using receptor-binding studies (Fehmann and Habener, 1991; Göke *et al*., 1992), northern blotting (Thorens *et al*., 1993), and immunohistochemistry (Hörsch *et al*., 1997; Tornehave *et al*., 2008; Pyke *et al*., 2014). These studies have provided evidence that pancreatic B cells are the target cells for GLP-1 in the mammalian species. However, our previous study demonstrated that the target cells of GLP-1 in the chicken pancreas are pancreatic D cells, which is different from that in mammals (Watanabe *et al*., 2014). The present study revealed that pancreatic D cells in northern bobwhites and ostriches also express GLP-1R. These data suggest that GLP-1 function might be mediated by somatostatin secreted from pancreatic D cells via GLP-1R in the avian species. Fehmann and Habener (1991) have demonstrated that pancreatic B and D

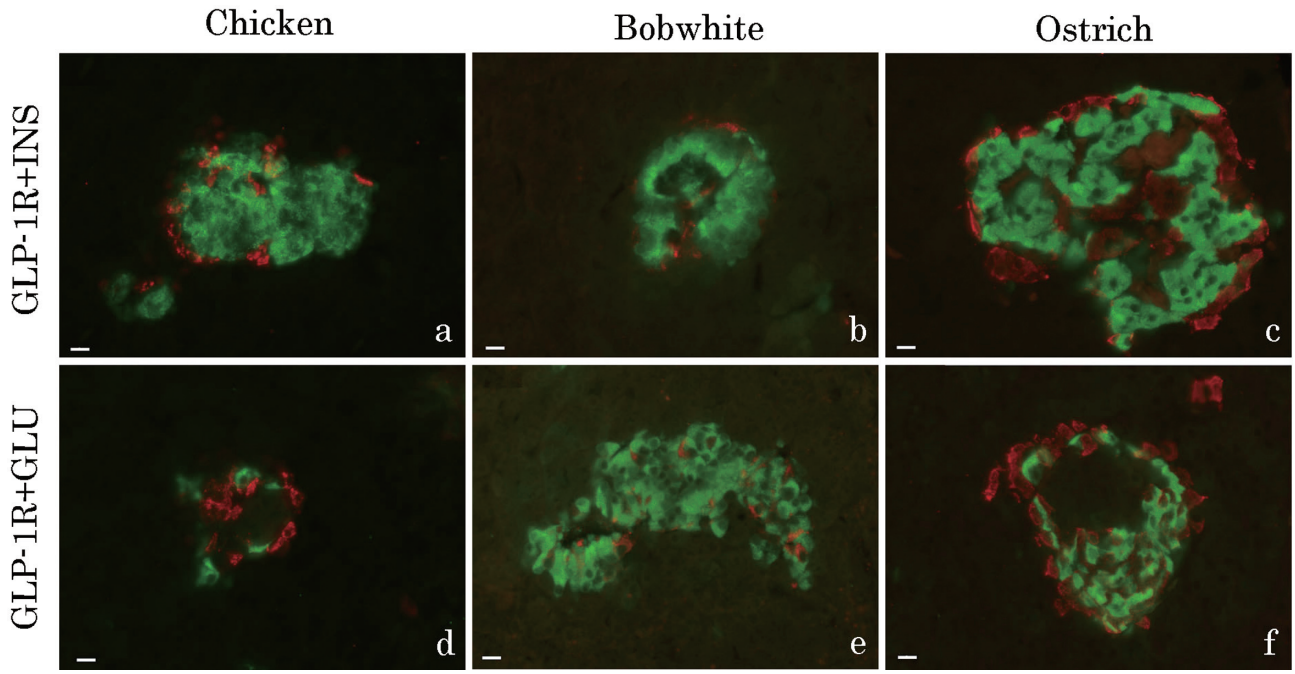

Fig. 2. **Merged images of double immunofluorescence pictures of glucagon-like peptide-1 receptor (red) and insulin (green) (GLP-1R + INS, a–c), and glucagon-like peptide-1 receptor (red) and glucagon (green) (GLP-1R + GLU, d–f) in the pancreatic islets of chickens (a and d), northern bobwhites (b and e), and ostriches (c and f).** Islet cells showing either insulin or glucagon immunoreactivity were immunonegative to glucagon-like peptide-1 receptor. Bars indicate 10 μm.

cells express GLP-1R, and insulin and somatostatin secretion induced by GLP-1(7–37) suppresses glucagon secretion. On the other hand, Tornehave *et al*. (2008) have shown that GLP-1R expression is restricted to B cells in the mouse, rat, and human pancreases. In the present study, cGLP-1R immunoreactivity was found to be absent in the pancreatic B cells of chickens, northern bobwhites, and ostriches. Hence, it is probable that pancreatic D cells, instead of B cells, are the target cells for the avian GLP-1.

Based on the composition of three endocrine cells; A, B, and D cells, ultrastructural and immunohistochemical studies have shown that avian pancreas contains several types of pancreatic islets. Three types of pancreatic islets; A, B, and mixed (mammalian type), have been found in many avian species including chickens and Japanese quails (Smith, 1974; Watanabe *et al*., 1975; Schwarz *et al*., 1983; Mikami *et al*., 1985; Lucini *et al*., 1996). Weir *et al*. (1976) have shown that somatostatin immunoreactivity in the chicken pancreas was 21 times higher than that in the rat. Watanabe *et al*. (1990–1991) demonstrated that five types of islets are present in the ostrich pancreas and all types of pancreatic islets contain D cells. Moreover, ostrich islets contain more D cells than those present in other avian species (our unpublished data). These data suggest that somatostatin plays an important role in the regulation of blood glucose levels in avian species.

In the present study, we demonstrated that D cells in all types of pancreatic islets exhibited GLP-1R immunoreactivity in three avian species, which are taxonomically different from one another. Therefore, GLP-1R expression in the pancreatic D cells might be a common phenomenon in avian species. Moreover, morphological and immunocytochemical studies revealed that chicken pancreatic D cells are innervated by cholinergic nerves originating from the vagus nerve (Watanabe and Fujioka, 1980; Hiramatsu *et al*., 1988). Such present and past findings suggested that the secretory function of pancreatic D cells is regulated by neural and endocrine systems in avian species.

The present study involving white leghorn chicken, northern bobwhite, and common ostrich concluded that pancreatic D cells are the target cells of GLP-1, and GLP-1 plays a part of its physiological roles via somatostatin in avian species.

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