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# Expression of proglucagon and proglucagon-derived peptide hormone receptor genes in the chicken ☆

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**Abstract**

To better understand how the proglucagon system functions in birds, we utilized a molecular cloning strategy to sequence and characterize the chicken proglucagon gene that encodes glucagon, glucagon-like peptide (GLP)-1 and GLP-2. This gene has seven exons and six introns with evidence for an additional (alternate) first exon and two promoter regions. We identified two distinct classes of proglucagon mRNA transcripts (PGA and PGB) produced by alternative splicing at their 3′-ends. These were co-expressed in all tissues examined with pancreas and proventriculus showing the highest levels of each. Although both mRNA classes contained coding sequence for glucagon and GLP-1, class A mRNA lacked that portion of the coding region (CDS) containing GLP-2; whereas, class B mRNA had a larger CDS that included GLP-2. Both classes of mRNA transcripts exhibited two variants, each with a different 5′-end arising from alternate promoter and alternate first exon usage. Fasting and refeeding had no effect on proglucagon mRNA expression despite significant changes in plasma glucagon levels. To investigate potential differences in proglucagon precursor processing among tissues, mRNA expression for two prohormone convertase (PC) genes was analyzed. PC2 mRNA was predominantly expressed in pancreas and proventriculus, whereas PC1/3 mRNA was more highly expressed in duodenum and brain. We also determined mRNA expression of the specific receptor genes for glucagon, GLP-1 and GLP-2 to help define major sites of hormone action. Glucagon receptor mRNA was most highly expressed in liver and abdominal fat, whereas GLP-1 and GLP-2 receptor genes were highly expressed in the gastrointestinal tract, brain, pancreas and abdominal fat. These results offer new insights into structure and function of the chicken proglucagon gene, processing of the precursor proteins produced from it and potential activity sites for proglucagon-derived peptide hormones mediated by their cognate receptors.
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## 1. Introduction

Glucagon and two glucagon-like peptides (GLP-1 and GLP-2) comprise the predominant members of a group of peptides that are derived from a common precursor protein via proteolytic processing. In mammals, the proglucagon precursor is encoded by a single gene which expresses a single mRNA (Kieffer and Habener, 1999). Analysis of mammalian proglucagon gene sequence and structure revealed that glucagon, GLP-1 and GLP-2 are each encoded by a separate exon (Heinrich et al., 1984; White and Saunders, 1986). This genomic organization suggests that the three peptide hormones evolved from ancestral glucagon-like sequence as a result of exon duplication (Irwin, 2001). Non-mammalian vertebrates have been reported to exhibit more complex mechanisms for proglucagon gene regulation involving tissue-specific mRNA splicing and differing coding potential of duplicate genes

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that give rise to multiple proglucagon mRNA transcripts and precursor proteins (Irwin, 2001).

Post-translational proteolytic processing of the proglucagon precursor protein is mediated by prohormone convertase (PC) enzymes and this mechanism is responsible for the tissue-specific production of a variety of proglucagon-derived peptides in mammals (Kieffer and Habener, 1999; Sinclair and Drucker, 2005). The expression of specific PC genes (e.g., PC1/3, PC2) and the resulting proteolytic activity of these enzymes in different tissues determine how the proglucagon precursor is processed. For example, the production of glucagon from proglucagon in pancreatic islet α-cells requires the action of PC2, whereas PC1/3 is essential for the nutrient-dependent production of GLP-1 and GLP-2 by enteroendocrine cells in the intestine and selected neurons within the brain (Dey et al., 2005; Dhanvantari et al., 1996; Kieffer and Habener, 1999; Rouille et al., 1995; Sinclair and Drucker, 2005).

Glucagon, a 29 amino acid peptide hormone produced by the α-cells of the pancreatic islets in response to reduced levels of blood glucose, plays an important counter-regulatory role to insulin in maintaining glycemic control and energy balance through its effects on glucose, lipid and amino acid metabolism (Jiang and Zhang, 2003). Glucagon-like peptide-1 is an incretin hormone that regulates blood glucose and promotes glucose homeostasis by stimulating pancreatic insulin synthesis and secretion, and islet cell proliferation and neogenesis while inhibiting the secretion of glucagon (Drucker, 2001). In addition, GLP-1 controls nutrient absorption through its inhibitory effects on gastric emptying and food intake regulation (Kieffer and Habener, 1999). Potential functions for GLP-2 have only been reported for mammalian species in which it is thought to play a role in intestinal growth and nutrient absorption by maintaining the integrity of epithelial cells (Burrin et al., 2003; Drucker, 2001). Secreted by enteroendocrine L-cells in response to the presence of intestinal nutrients, GLP-2 has been found to promote crypt cell proliferation and suppress apoptosis in mucosal epithelial cells (Burrin et al., 2003; Estall and Drucker, 2006).

The physiological effects of glucagon, GLP-1 and GLP-2 on regulating metabolism are mediated by a specific set of related G-protein-coupled receptors (GPCRs) belonging to the glucagon–secretin receptor class II family of GPCRs (Mayo et al., 2003). Genes encoding each of these receptors have been identified, cloned and sequenced for a number of species and their expression in different tissues has been characterized (Irwin, 2005; Irwin and Wong, 2005; Kieffer and Habener, 1999; Mayo et al., 2003). Moreover, the unique functions and specific binding characteristics of each of these receptors have been extensively studied in mammals using native hormone ligands as well as specific agonists and antagonists (Drucker, 2001; Kieffer and Habener, 1999; Sinclair and Drucker, 2005). It has been postulated that receptor–ligand specificity evolved separately following duplication of the glucagon-like peptides and their corresponding receptors (Irwin and Wong, 2005; Irwin, 2005). This conclusion is supported by the observation that, in fish, GLP-1 exhibits biological activity similar to glucagon due to its binding by a receptor that possesses signaling characteristics resembling the glucagon receptor rather than the GLP-1 receptor (Irwin and Wong, 2005; Plisetskaya and Mommsen, 1996).

Compared to mammalian species, there has been relatively little investigation of the proglucagon-derived peptides and their cognate receptors in birds. Previous studies in chickens, which posses a single proglucagon gene, found two different cDNA clones isolated from pancreatic and intestinal cDNA libraries (Hasegawa et al., 1990; Irwin and Wong, 1995). The pancreatic cDNA encoded glucagon and GLP-1, but not GLP-2; whereas, the intestinal cDNA contained coding sequence for all three peptides. These observations suggested that regulation of chicken proglucagon gene expression involves tissue-specific mRNA splicing in addition to the downstream post-translational proteolytic processing that determines which peptides are produced from the precursor and ultimately secreted (Yue and Irwin, 2005). Although glucagon and GLP-1 appear to function similarly in both birds and mammals (Hazelwood, 1984; Honda et al., 2007; Shousha et al., 2007; Tachibana et al., 2004, 2006, 2007), as of yet, there have been no reports of a physiological role for GLP-2 in birds. Moreover, there are only a limited number of reports in chickens on the expression of proglucagon mRNA and GLP-1 immunoreactive staining in brain and ocular tissues (Feldkaemper et al., 2004; Tachibana et al., 2005), glucagon receptor mRNA in the retina (Buck et al., 2004) or indirect evidence for functional GLP-1 receptors using agonists and antagonists of GLP-1 such as N-terminal GLP-1 peptide fragments or exendin 5–39 (Furuse et al., 1998; Tachibana et al., 2001). Despite the fact that the draft chicken genome contains requisite sequence for proglucagon and proglucagon-derived peptide receptor genes (Irwin and Wong, 2005; Yue and Irwin, 2005), there has been no systematic attempt to characterize the expression of these genes in different chicken tissues. Therefore, the objectives of this study were to: (1) further characterize the chicken proglucagon gene structure and sequence; (2) to determine possible mechanisms contributing to the expression of multiple proglucagon mRNA transcripts and precursor protein processing in different tissues; and (3) to study the expression of proglucagon-related peptide receptor genes in different tissues.

## 2. Materials and methods

### 2.1. Animals, tissues and nucleic acid isolation

Male broiler chicks (*Gallus gallus*) were reared from day of hatch to 3 wks of age in heated battery/brooder units. All birds received a standard starter poultry ration and water *ad libitum*. At 3 wks, tissue samples were collected, snap frozen in liquid nitrogen and stored at −80 °C prior to RNA isolation. In a separate experiment, 30 broiler chickens (3-wk-old males) were divided into five groups of six birds each and subjected to the following treatments: (1) fed *ad libitum*, (control), (2) fasted for 24 h