General and Comparative Endocrinology 209 (2014) 50–60

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Evolution of receptors for peptides similar to glucagon

David M. Irwin *

Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ont. M5S 1A8, Canada
Banting and Best Diabetes Centre, University of Toronto, Toronto, Ont. M5S 1A8, Canada

ARTICLE INFO

Article history:
Available online 17 March 2014

Keywords:
Glucagon
Glucagon-like peptides
Glucose-dependent insulinotropic peptide (GIP)
Receptors
Evolution
Exendin

ABSTRACT

The genes encoding the peptide precursors for glucagon (*GCG*), glucose-dependent insulinotropic peptide (*GIP*), and ortholog of exendin belong to the same family as shown by sequence similarity. The peptides similar to glucagon encoded by these genes signal through a closely related subfamily of G-protein coupled receptors. A total of five types of genes for receptors for these peptides have been identified, three for the products of *GCG* (*GCGR*, *GLP1R*, and *GLP2R*) and one each for the products of *GIP* (*GIPR*) and the ortholog of exendin (*Grlr*). Phylogenetic and genomic neighborhood analyses clearly show that these genes originated very early in vertebrate evolution and all were present in the common ancestor of tetrapods and bony fish. Despite their ancient origins, some of these genes are dispensable, with the *Glp1r*, *Gipr*, and *Grlr* being lost on the lineages leading to bony fish, birds, and mammals, respectively. The loss of the genes for these receptors may have been driving forces in the evolution of new functions for these peptides similar to glucagon.

1. Proglucagon and secretin-like peptides

The proglucagon (*GCG*) gene encodes hormones that have essential and differing roles in mammalian physiology. The three major hormones encoded by proglucagon are glucagon and the two glucagon-like peptides glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) (Kieffer and Habener, 1999; Drucker, 2005). Glucagon is the counter-regulatory hormone to insulin and induces glucose production by the liver when blood glucose levels are low (Jiang and Zhang, 2003; Ramnanan et al., 2011). GLP-1 is a major incretin hormone that potentiates insulin release by pancreatic islet beta cells in response to eating a meal (Meier and Nauck, 2005; Holst, 2007; Baggio and Drucker, 2007). GLP-2 has important roles in maintaining intestinal function (Drucker, 2001; Baggio and Drucker, 2007). The major actions of these hormones are non-overlapping and demonstrate the diverging functions of related sequences (Kieffer and Habener, 1999; Drucker, 2005). In addition to these major physiological functions, these three glucagon-like hormones produced from proglucagon have additional important activities, many of which are involved in feeding behavior (Alvarez et al., 1996; Lovshin et al., 2004; Baggio and Drucker, 2007). The processing of proglucagon to produce the glucagon-like hormones also yields additional peptides, e.g., intervening peptide-1 (IP1), which may have additional physiological functions (Kieffer and Habener, 1999; Drucker, 2005).

The proglucagon-derived hormones are members of a larger family of secretin-like hormones that are found to play diverse physiological roles in many metazoan species (Hoyle, 1998; Sherwood et al., 2000; Roch et al., 2009). Mammalian genomes contain six genes that encode a total of 10 secretin-like hormones (Hoyle, 1998; Sherwood et al., 2000; Roch et al., 2009). In addition to the proglucagon (*GCG*) gene, which encodes three secretin-like sequences (glucagon, GLP-1 and GLP-2), the adenylate cyclase activating peptide (*ADCYAP*) and vasoactive intestinal peptide (*VIP*) genes each encode two secretin-like sequences. *ADCYAP* encodes the hormones PACAP (pituitary adenylate cyclase activating protein) and PACAP-related peptide (PRP; sometimes called growth hormone releasing hormone-like peptide, GHRH-LP) while *VIP* encodes VIP and peptide histidine methionine (PHM) or peptide histidine isoleucine (PHI) (depending upon whether last amino acid of the hormone in the species is methionine or isoleucine). The three remaining human genes each encode a single secretin-like sequence, and they are the genes for secretin (*SCT*), growth hormone releasing hormone (*GHRH*) and glucose-dependent insulinotropic peptide (*GIP*). Additional secretin-like sequences have been identified in some vertebrate species including the exendins (Hoyle, 1998), which were first identified in the reptile Gila monster and relatives (*Heloderma suspectum* and *Heloderma horridum*)

* Address: Department of Laboratory Medicine and Pathobiology, University of Toronto, Room 6211 Medical Sciences Building, 1 King's College Circle, Toronto, Ont. M5S 1A8, Canada.
E-mail address: david.irwin@utoronto.ca

http://dx.doi.org/10.1016/j.ygcen.2014.03.002
0016-6480/

(Raufman, 1996). Recently putative orthologs of the gene encoding exendin have been identified in other vertebrates, including other reptiles, birds, amphibians and fish, but not in mammals (Irwin and Prentice, 2011; Irwin, 2012; Wang et al., 2012; Park et al., 2013). This gene has also been called glucagon-like (*Gcgl*) (Wang et al., 2012) and glucagon-related peptide (*Gcrp*) (Park et al., 2013).

Phylogenetic analysis of the secretin-like hormones yields poorly supported trees due to the short length of their peptide sequences (Dores et al., 1996). However, phylogenetic analyses typically show that the secretin-like peptides encoded by the proglucagon (glucagon, GLP-1 and GLP-2) and GIP genes are most closely related (Hoyle, 1998; Irwin et al., 1999; Cardoso et al., 2010; Ng et al., 2010; Irwin, 2012) (see Fig. S1). Exendin-1 and -2, from the Gila monster, are most similar to GLP-1, while exendin-3 and -4 are very divergent and more similar to other secretin-like hormones (Hoyle 1998; Sherwood et al., 2000; Fry et al., 2010). Comparison of the exendin-1 and -4 precursor sequences, although, showed that the non-hormone portion of the precursors (signal and N-terminal pro-peptide sequences) are very similar (Pohl and Wank, 1998). These observations suggested that the exendin peptides experienced convergent evolution, with some of the exendin sequences evolving to be more similar to either GLP-1 or VIP (Fry et al., 2010). The inclusion of putative orthologs of the exendin peptides (from other reptiles and birds) into phylogenetic analyses of secretin-like peptides allowed a better resolution of the relationships of exendin to the other secretin-like peptides revealing that exendin is most closely related to the proglucagon-derived peptides and GIP (Irwin and Prentice, 2011; Irwin, 2012; Wang et al., 2012; Park et al., 2013) (see Fig. S1). This recent phylogenetic analysis of secretin-like peptides concludes that exendin-1 and -2 evolved convergently upon the VIP sequence (Irwin, 2012). The close relationship of the exendin, *GCG*, and *GIP* genes was strengthened by the observation that the genomic location of the exendin ortholog is similar to those for the *GCG* and *GIP* genes (Irwin and Prentice, 2011; Irwin, 2012; Wang et al., 2012; Park et al., 2013). These observations suggest that *GCG*, *GIP*, and the gene for the exendin ortholog represent three of the four genes generated during the pair of genome duplications that occurred on the ancestral vertebrate lineage (Irwin and Prentice, 2011; Irwin, 2012; Wang et al., 2012). The remaining genes encoding the secretin-like sequences are not found in similar genomic neighborhoods, consistent with a conclusion that the *GCG*, *GIP*, and exendin ortholog genes are more closely related to each other than they are to any of the other secretin-like genes (Irwin and Prentice, 2011).

2. Genes for the receptors for peptides similar to glucagon

Proglucagon-derived peptides and other secretin-like hormones exert their physiological effects through binding to specific receptors found on the surfaces of cells in the target tissues. A cDNA clone for a specific receptor for the GLP-1 receptor (GLP1R) was cloned in 1992 and found to encode a G-protein coupled receptor (GPCR) (Thorens, 1992). Receptors for the other two glucagon-like peptides encoded by *GCG*, glucagon (GCGR) and GLP-2 (GLP2R), are also G-protein coupled receptors (Jelinek et al., 1993; Munroe et al., 1999), as are the receptors for the peptide similar to glucagon encoded by the *GIP* gene (GIPR; Usdin et al., 1993) and the putative ortholog of Gila monster exendin (GRLR, Irwin and Prentice, 2011; also called GCGLR, Wang et al., 2012; and GCRPR, Park et al., 2013). The genes for each of these receptors are expressed in a tissue-specific manner, with high expression in the physiologically relevant tissues (i.e., tissues where the hormones have known or predicted physiological action). While the function of the ortholog of exendin is currently unknown, the observation that the *Grlr* gene is most abundantly expressed in the brain (Irwin and Prentice, 2011; Wang et al., 2012) suggests that the likely physiological target of the exendin ortholog is in the brain, however, this still leaves a long list of potential functions. The discovery that *GLP1R* and *GLP2R* are also expressed in portions of the brain (Alvarez et al., 1996; Lovshin et al., 2004) prompted searches for additional functions for these peptides. Receptors for the peptides similar to glucagon range in size from about 400 to 550 amino acids in length, with GIPR being shortest and GLP2R being longest (see Figs. 1 and S2). Alignment of the protein sequences of these receptors show that the sequences show similarity across most of their length, with the regions encoding the seven transmembrane domain regions being well-conserved, as are the distances between these seven domains, whereas the N-terminal and C-terminal regions show greater variability both in length and in their sequences (see Figs. 1 and S2).

The genes for the receptors for peptides similar to glucagon are dispersed on three human chromosomes, with two on chromosome 17 (*GCGR* and *GLP2R*) and one each on chromosomes 6 (*GLP1R*) and 19 (*GIPR*) (see Table S1). The exon–intron gene structures of these genes are similar, as are the genes for other secretin-like hormone receptors, with the protein-coding region distributed over 13 coding exons (Fig. 1). The structure of the *GCGR* and *GIPR* genes differ from those of the *GLP1R* and *GLP2R* by having an additional upstream exon that only contains 5′ untranslated sequence (Fig. 1). Introns between the coding exons are at very similar positions in the alignment of the receptor protein sequences, with the homologous introns interrupting the coding sequences in identical phases (Figs. 1 and S2). With the exception of the first and last coding exons, the lengths of the remaining coding exons are similar among the receptor genes (Table S2).

In parallel with the identification of the orthologs of the Gila monster exendin gene, an additional glucagon receptor-like gene, the glucagon receptor-like receptor (*Grlr*, also called *Gcglr* and *Gcrpr*) was found in the genome sequence of many vertebrate species (Irwin and Prentice, 2011; Wang et al., 2012; Park et al., 2013). The initial study identifying this receptor gene (Irwin and Prentice, 2011) hypothesized that the product of the exendin ortholog should be the ligand for this receptor, with subsequent studies confirming this interaction (Wang et al., 2012; Park et al., 2013). The human genome, like that of other mammals with available genome sequences, does not contain a *GRLR* gene, and to date this gene has only been found in non-mammalian vertebrates (Irwin and Prentice, 2011; Wang et al., 2012; Park et al., 2013). The intron–exon structure of the *Grlr* gene, as well as the sizes of its coding exons, is similar to those for the genes for other receptors for peptides similar to glucagon (Fig. 1).

3. Receptors for peptides similar to glucagon and Class B1 of G-protein coupled receptors

Receptors for peptides similar to glucagon encoded by *GCG*, *GIP*, and the ortholog of the Gila monster exendin gene are GPCRs, as are the receptors for many other secretin-like peptides. Specific receptors for secretin (SCTR), VIP ($VPAC_1$ and $VPAC_2$), GHRH (GHRHR) and PACAP (ADCYAP1R1) are all GPCRs (see Table S1) (Fredriksson et al., 2003; Fredriksson and Schiöth, 2005). The large GPCR gene family consists of at least 850 genes in mammals (Fredriksson et al., 2003; Fredriksson and Schiöth, 2005; Bjarnadóttir et al., 2006; Krishnan et al., 2013). Phylogenetic analyses of GPCR genes in a number of vertebrate species have consistently identified a subset of GPCRs, designated the Class B1 GPCRs, which include the receptors for the secretin-like peptides described above as well as receptors for corticotropin releasing hormone (CRHR1 and CRHR2), parathyroid (PTH1R and PTH2R), calcitonin (CALCR) and a calcitonin receptor-like gene (CALCRL) (Harmar, 2001; Fredriksson et al., 2003; Fredriksson and Schiöth, 2005;