General and Comparative Endocrinology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Research paper

Pancreatic PYY but not PPY expression is responsive to short-term nutritional state and the pancreas constitutes the major site of PYY mRNA expression in chickens

Angus M.A. Reid [a,*], Peter W. Wilson [a], Sarah D. Caughey [a], Laura M. Dixon [b], Rick B. D'Eath [b], Victoria Sandilands [c], Timothy Boswell [d], Ian C. Dunn [a]

[a] Roslin Institute, University of Edinburgh, EH25 9RG, Scotland, United Kingdom
[b] Scotland's Rural College (SRUC), Edinburgh EH9 3JG, Scotland, United Kingdom
[c] SRUC Avian Science Research Centre, Auchincruive, KA6 5HW, Scotland, United Kingdom
[d] School of Biology, Newcastle University, NE1 7RU, England, United Kingdom

ARTICLE INFO

Article history:
Received 24 February 2017
Revised 23 June 2017
Accepted 5 July 2017
Available online xxxx

Keywords:
Peptide YY
Pancreatic polypeptide
Energy homeostasis
Feeding
Broiler

ABSTRACT

PP-fold peptides such as peptide YY (PYY) and pancreatic polypeptide (PPY) are known to play key roles in vertebrate energy homeostasis. Until recently, no gene sequence was available for avian PYY and therefore a gap in knowledge of regulation of its expression exists in avian species. Here we further evidence the mRNA sequence for chicken PYY and show that the pancreas is the major site of its mRNA expression, with a secondary peak of expression around the distal jejunum, in contrast to mammals where the large intestine is the major site of PYY expression. We also demonstrate that pancreatic PYY expression is responsive to short-term and long-term nutritional state, increasing within hours of feeding, in contrast to intestinal PYY which does not fluctuate to the same extent, and pancreatic PPY which appears to be primarily determined by long-term energy state. Both pancreatic PYY and PPY expression were found to exhibit ontogeny, being evenly distributed throughout the pancreas in young (2wk) chicks but having a decreasing splenic to duodenal gradient by adolescence (12wk).

1. Introduction

Peptide YY (PYY) is one of three known members of the PP-fold family of proteins, along with neuropeptide Y (NPY) and pancreatic polypeptide (PPY) (Cerda-Reverter and Larhammar, 2000). The structure and function of the PYY gene is relatively well-documented in mammals (Batterham and Bloom, 2003; McGowan and Bloom, 2004; Ueno et al., 2008); however, very little transcriptional work has been reported in avian species. This is due to the lack of a gene sequence, despite early elucidation of the peptide sequence (Conlon and Oharte, 1992) and relatively high conservation of the peptide (Blomqvist et al., 1992). The most recent chicken genome build – Gallus_gallus-5.0, GenBank accession GCF_000002315.4 – erroneously annotated a predicted PYY gene sequence at position KQ759583.1: 13,290–14,841 (Ensembl) but this is in fact the chicken PPY gene, encoding pancreatic polypeptide (Nata et al., 1993). The first articles evidencing the true chicken PYY mRNA sequence were recently published (Aoki et al., 2017; Gao et al., 2017) but these disagree on the transcriptional start and termination sites. Issues in definitively characterising the transcript may be due to homology with the other PP-fold gene family members and regions of high GC content, a feature of PYY genes across taxa (e.g. human PYY mRNA (NM_004160.5) region 940–1020 = 77.8% GC content, lizard PYY (XM_003222643.3) region 400–444 = 71.1% GC).

In vertebrates, peripheral PYY is purported to act as a satiety factor released from the gastrointestinal tract after feeding to curb appetite via afferent vagal Y-receptors or directly within the arcuate nucleus of the hypothalamus (Batterham and Bloom, 2003; Batterham et al., 2002; Simpson et al., 2012; Ueno et al., 2008). PYY-expressing neurones also exist in the brain in mammals and fish, however some contention exists as to the relative levels of PYY expression in different tissues and their functional significance. In goldfish for example, central PYY is reported to be more highly expressed than intestinal PYY and is upregulated in satiety, in contrast to observations for other vertebrates including other fish (Murashita et al., 2009; Wall and Volkoff, 2013). Discrete populations of PYY-expressing cells have been identified throughout the brain (Bottcher et al., 1985; Cerda-Reverter et al., 2000) and

* Corresponding author.
E-mail address: angus.reid@roslin.ed.ac.uk (A.M.A. Reid).

http://dx.doi.org/10.1016/j.ygcen.2017.07.002
0016-6480/

digestive system (Ekblad and Sundler, 2002; El-Salhy et al., 1983), so opposing or unrelated roles for PYY expressed from distinct regions might account for inconsistencies in whole-tissue measurements, along with interspecific variation and differences in satiety state. In tetrapods at least, the accepted dogma is that peripheral PYY acts as an anorectic factor released from the intestine after meals to curb food intake and modulate gastrointestinal function, whereas central PYY has a functionally opposite orexigenic effect (McGowan and Bloom, 2004; Ueno et al., 2008).

Distinct binding preferences and tissue distributions of several Y-receptors (Dumont et al., 1998; Keire et al., 2002; Larhammar, 1996) allude to the broad range of biological functions attributed to central and peripheral PP-fold peptide activity. Discrete ligand expression and variable receptor specificity conferred by proteolytic processing of ligands (Cerda-Reverter et al., 2000; Mentlein et al., 1993) likely further facilitate diverse and dynamic endocrine and paracrine roles for PP-fold peptides *in vivo*. Chicken PYY is known to differ in primary structure to mammalian PYY, resulting in anomalous cleavage of the signal peptide and an elongated 37-residue ligand apparently impervious to proteolysis by DPP4 (Conlon and Oharte, 1992). This theoretically restricts posttranslational variability of receptor specificity and implies the possibility of alternative modes of PYY action in the chicken (and perhaps across avian species) but the biological implications of proteolysis are not comprehensively understood for any species. Since chicken PYY remains under-studied, describing the tissue distribution and nutritional state-responsiveness of its expression is a priority to inform future studies investigating the precise biological roles of PYY in birds.

PPY was originally identified in the chicken (Kimmel et al., 1975) and is known to be predominantly expressed in the pancreas in vertebrates (Ekblad and Sundler, 2002; Gao et al., 2017). PPY is relatively well-characterised as a satiety hormone, with increased circulating PPY observed within minutes to hours after feeding in avian (Johnson and Hazelwood, 1982) and mammalian (Asakawa et al., 2003) models. Exogenous PPY was also shown to reduce food intake in a dose-dependent manner after intraperitoneal administration in mice (Asakawa et al., 2003), however no such peripheral injection studies have been carried out for avian PPY.

Defining the roles of PP-fold family members is important for understanding energy balance and growth in vertebrate species. This includes domestic fowl, for which there is a need to understand appetite regulation so that management of birds used for human food production might be optimised to maximise efficiency and ameliorate welfare concerns, such as restricted feeding in breeding meat-type birds (De Jong and Guemene, 2011). The few studies carried out on avian PYY have mostly involved application of exogenous PYY peptide and demonstrated an orexigenic effect of centrally-administered PYY (Ando et al., 2001; Kuenzel et al., 1987) and digestion- and growth-regulating effects of PYY applied systemically *in ovo* (Coles et al., 2001, 1999). Recent results from peripheral PYY administration and transcriptional measurements in feeding studies support the conserved role of intestinal PYY as an anorectic satiety factor in chickens (Aoki et al., 2017). Although PYY is traditionally considered an intestinal peptide, there is growing evidence that pancreas-derived PYY is important in regulation of digestion and satiety. Interesting ontogenic and regional gradient patterns of pancreatic expression of PYY and PPY have been demonstrated in mammals (Ekblad and Sundler, 2002; El-Salhy et al., 1983; Sandström and El-Salhy, 2002). Functional regulation of PYY expression has not yet been described in birds. In order to improve knowledge of peripheral control of satiety in birds and enable comparisons to be drawn when establishing conserved and divergent roles for PP-fold family peptides across taxa, we investigate in this study the tissue distribution of PYY and PPY mRNAs in the chicken and the effects of nutritional state on their expression, particularly in the pancreas.

2. Materials and methods

2.1. Sequence derivation

2.1.1. Avian PYY and PPY

The large amount of high-throughput sequencing information from the chicken (*Gallus gallus*) available in the sequence read archive (SRA) (Leinonen et al., 2011b) was mined to derive a contiguous mRNA sequence for chicken PYY. Relevant experiments were identified by using appropriate search terms at the European Nucleotide Archive (Leinonen et al., 2011a). Data files were then probed for PYY mRNA sequence using the known chicken PYY peptide sequence (AAB24283.1) as a query in tblastn (NCBI). Short read sequences of interest were downloaded in FASTA format and read into GAP (Bonfield and Whitwham, 2010) for alignment. The process was iterative and as each new consensus was built the SRA files were re-interrogated using nucleotide BLAST until no further 3′ or 5′ extension was obtained. The final consensus sequence was analysed for open reading frames using ExPasy Translate (Gasteiger et al., 2003) and likely signal peptide cleavage sites using SignalP (Petersen et al., 2011).

For confirmation of the cDNA 5′ end in chicken, rapid amplification of cDNA ends (RACE) was completed using the Roche 2nd generation 5′/3′ RACE kit as per the manufacturer's protocol and LightRUN Sanger sequencing (GATC Biotech) was employed to sequence the product. A similar data-mining process was followed for quail (*Coturnix coturnix*) PYY. This was only to increase confidence in inference to the evolving structures of vertebrate PYY and no further characterisation was carried out for quail PYY. The chicken PPY gene sequence was already definitively known (NM_204786.1) (Nata et al., 1993).

2.2. Animal experiments

Each animal experiment was approved by the Roslin Institute Animal Welfare and Ethical Review Body or SRUC Animal Ethical Committee, and compliant with UK Home Office legislation.

2.2.1. Distribution of expression of PYY and PPY across chicken tissues

In order to assess the distribution of expression of PYY and PPY in chicken tissues, four Ross 308 broilers raised in standard conditions were killed at 42 d and a broad range of tissue samples was collected.

2.2.2. Effects of short-term nutritional state on expression of PYY and PPY

Chicks from the 22nd generation of a pedigree broiler-layer hybrid line were reared under standard lighting (14 L:10 D) and temperature (26 °C ambient) conditions in one pen with *ad libitum* access to food until 10 days of age when they were separated into two experimental group pens (n = 12 per group) to balance sex and family. *Ad libitum* access to food was maintained until removal of food from both groups at 14 days of age, 2 h before lights-on. This was followed either by reintroduction of food after 3.5 h (*ad libitum* group, AL) or by maintenance of fasting conditions for a further 7–8 h to give a total fast duration of 10.5–11.5 h (fasted group, F). At the experimental endpoint, birds were killed by cervical dislocation and 40–100 mg samples from the pancreas head (splenic end) and tail (duodenal end) were immediately collected and snap-frozen on dry ice.

2.2.3. Effects of long-term nutritional state on expression of PYY and PPY at several timepoints after feeding

The general approach in testing different dietary regimes was based on a previously-described study (Dunn et al., 2013b). Broiler

Please cite this article in press as: Reid, A.M.A., et al. Pancreatic PYY but not PPY expression is responsive to short-term nutritional state and the pancreas constitutes the major site of PYY mRNA expression in chickens. Gen. Comp. Endocrinol. (2017), http://dx.doi.org/10.1016/j.ygcen.2017.07.002