What is Pancreatic Polypeptide and what does it do?

This document aims to evaluate current understanding of pancreatic polypeptide (PP), a gut hormone with several functions contributing towards the maintenance of energy balance. Successful regulation of energy homeostasis requires sophisticated bidirectional communication between the gastrointestinal tract and central nervous system (CNS; Williams *et al.* 2000). The coordinated release of numerous gastrointestinal hormones promotes optimal digestion and nutrient absorption (Chaudhri *et al.*, 2008) whilst modulating appetite, meal termination, energy expenditure and metabolism (Suzuki, Jayasena & Bloom, 2011).

The Discovery of a Peptide

Kimmel *et al.* (1968) discovered PP whilst purifying insulin from chicken pancreas (Adrian *et al.*, 1976). Subsequent to extraction of avian pancreatic polypeptide (aPP), mammalian homologues bovine (bPP), porcine (pPP), ovine (oPP) and human (hPP), were isolated by Lin and Chance (Kimmel, Hayden & Pollock, 1975). Following extensive observation, various features of this novel peptide witnessed its eventual classification as a hormone (Schwartz, 1983).

Molecular Structure

PP is a member of the NPY family including neuropeptide Y (NPY) and peptide YY (PYY; Holzer, Reichmann & Farzi, 2012). These biologically active peptides are characterized by a single chain of 36-amino acids and exhibit the same 'PP-fold' structure; a hair-pin U-shaped molecule (Suzuki *et al.*, 2011). PP has a molecular weight of 4,240 Da and an isoelectric point between pH6 and 7 (Kimmel *et al.*, 1975), thus carries no electrical charge at neutral pH.

Synthesis

Like many peptide hormones, PP is derived from a larger precursor of 10,432 Da (Leiter, Keutmann & Goodman, 1984). Isolation of a cDNA construct, synthesized from hPP mRNA, proposed that this precursor, pre-propancreatic polypeptide, comprised 95 residues (Boel *et al.*, 1984) and is processed to produce three products (Leiter *et al.*, 1985); PP, an icosapeptide containing 20-amino acids and a signal peptide (Boel *et al.*, 1984). PP is derived from the amino-terminus and the icosapeptide from the carboxy-terminus (**Fig. 1**) (Schwartz, 1983).

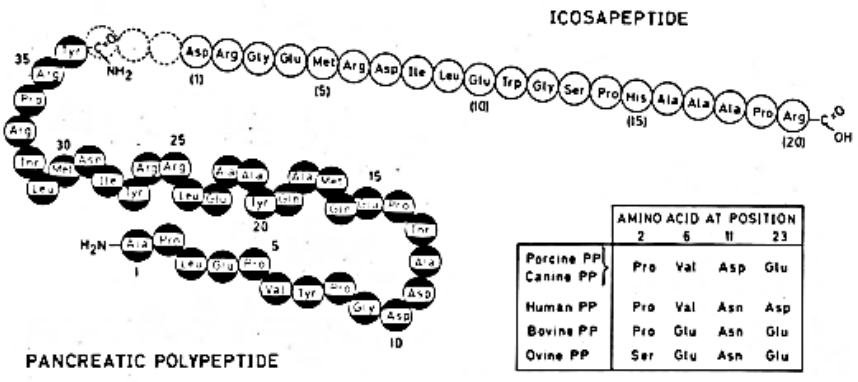

Figure 1. A schematic diagram illustrating the primary structure of pancreatic polypeptide (PP) and the icosapeptide synthesized within the common precursor hormone, pre-propancreatic polypeptide. The table (lower right) indicates differences in amino acid sequence found in five mammalian PP homologues (Schwartz, 1983).

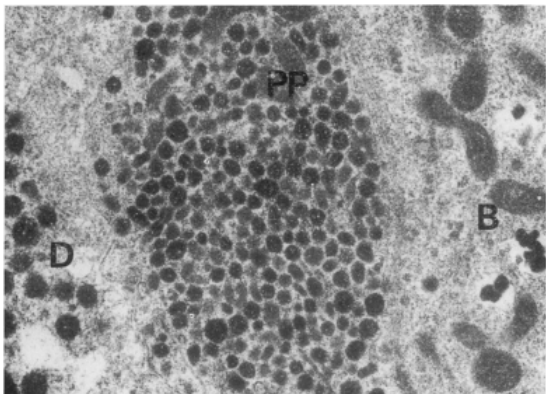

Figure 2. An electron micrograph image of pancreatic tissue showing a PP cell (PP) situated within an islet of Langerhan's, an insulin-producing β-cell (B) and a somatostatin-producing δ-*cell (D) (Adrian, 1978).*

Secretion

PP is predominantly secreted by the PP cells, also known as 'F cells' (Koska *et al*., 2004), dominating the duodenal pancreas (Schwartz, 1983). Minor amounts are also secreted by colonic and rectal cells of the distal gut (Adamska *et al*., 1994). Most PP cells are located at the periphery of the endocrine islets of Langerhans (**Fig. 2**) with fewer present within the exocrine acinar cells (Wynne *et al*., 2005).

Stimulatory Mechanisms

The principal stimulus for PP secretion is parasympathetic vagal cholinergic innervation (Dembiński *et al*., 2004). Vagotomy reaffirms this as ablation of the vagal nerves attenuates PP secretion. Administration of atropine, a competitive muscarinic acetylcholine antagonist (Kumari, Sreetama & Mohanakumar, 2007), also eliminates PP response (Schwartz, 1983), demonstrating that release is governed by cholinergic mechanisms (**Fig. 3**). Consequently, basal PP levels correspond to parasympathetic activity (Tong *et al*., 2007). Concentrations fluctuate diurnally measuring lowest in the morning and peaking during the evening (Wynne *et al*., 2005). Also, secretion increases with age, quadrupling between 30 and 70 years old, reflecting heightened vagal tone (Schwartz, 1983).

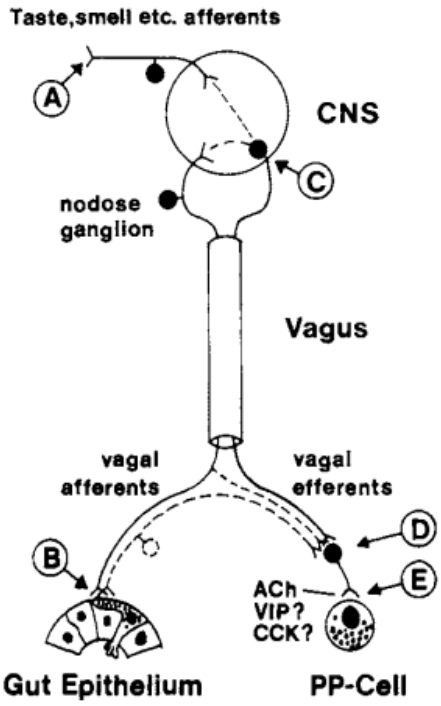

Figure 3. A schematic diagram showing parasympathetic vagal innervation of the pancreatic PP cell. A) Gustatory and olfactory sensations stimulate cranial-nerve afferent signal transmission to the central nervous system (C). In response, vagal efferent neurons stimulate pancreatic polypeptide (PP) release. B) Activation of gut epithelial mechanoreceptors upon gastric distension induces vagal afferent stimulation and PP secretion. D) Bioactive peptides such as cholecystokinin (CCK) and vasoactive intestinal polypeptide (VIP) modulate the postganglionic efferent neurone, stimulating PP release. Infusion of other peptides also regulates secretion. E) Release of acetylcholine (Ach) neurotransmitter from vagal nerves is the major stimulus to PP secretion (Schwartz, 1983).

PP is mainly released in response to food intake (Batterham *et al*., 2003) proportional to caloric consumption (Adamska *et al*., 1994). Secretion is biphasic (Wynne *et al*., 2005) with a cephalic and gastrointestinal phase (Schwartz, 1983). The initial cephalic phase is represented by a rapid postprandial rise in plasma PP shortly after eating, the rate of which surely depends on neural mechanisms. Sham-feeding, a process whereby food ingested does not reach the stomach, triggers this cephalic phase (Schwartz, 1983). Gustatory and olfactory sensations are responsible for transmission of neural afferent signals to the CNS with vagal efferent neurones subsequently stimulating PP release (**Fig. 3**). The secondary gastrointestinal phase is stimulated by various enteric stimuli, primarily protein and lipid consumption (Schwartz, 1983) and is prolonged with plasma PP concentrations remaining elevated for up to 6 hours post-satiation (Jesudason *et al*., 2007). Food entering the stomach causes gastric distension which mediates food intake (Wang *et al*., 2008). Activation of gut epithelial mechanoreceptors induces vagal afferent stimulation of PP cells thus PP release (**Fig. 3**) (Troke, Tan & Bloom, 2014).

Duodenal entry of pancreaticobiliary juice also stimulates secretion (Dembiński *et al.*, 2004). Other secretagogues include bioactive peptides; gastrin, gastrin releasing peptide (GRP), secretin (Dembiński *et al.*, 2004), ghrelin, motilin (Wynne *et al.*, 2005) vasoactive intestinal polypeptide (VIP) and cholecystokinin (CKK; **Fig. 3**; Schwartz, 1983). Hypoglycaemia, which can be induced by insulin infusion, is another powerful stimulus. The extent of secretion is proportional to the decrement in blood glucose (Schwartz, 1983). During exercise, endogenous sympathetic adrenergic mechanisms provoke mild PP secretion. This is markedly lower than observed following meal consumption (Schwartz, 1983). Conversely, hyperglycaemia; which can be induced by glucose infusion, inhibits PP secretion. Peptide hormones; somatostatin and bombesin, glucocorticoids, elevated plasma fatty acids and morphine are examples of PP inhibitors (Schwartz, 1983).

Receptor Interactions

PP-fold peptides elicit their effects through interaction with G-protein-coupled receptors; Y1 to Y6 (Sliwińska-Mossoń, Borowiecka & Milnerowicz, 2012). These seven-transmembrane-domain receptors are distributed within central tissues; the hypothalamus and brainstem (Troke *et al.*, 2014). PP, unable to diffuse across the blood-brain barrier, interacts with Y4 and Y5 receptors (Adamska *et al.*, 1994) but is the most potent agonist for Y4 receptors (Cabrele & Beck-sickinger, 2000) located within the hypothalamic arcuate nucleus (ARC) or dorsal vagal complex (DVC) of the brainstem. The brainstem, a principal region for PP activity, comprises neuronal populations crucial for appetite regulation (Troke *et al.*, 2014); the dorsal motor nucleus of vagus (DVN), area postrema (AP) and nucleus of the tractus solitarius (NTS; **Fig. 4**; Suzuki *et al.*, 2011). Circulating PP can access the DVN and bind its respective receptor to modulate energy balance (Asakawa *et al.*, 2003).

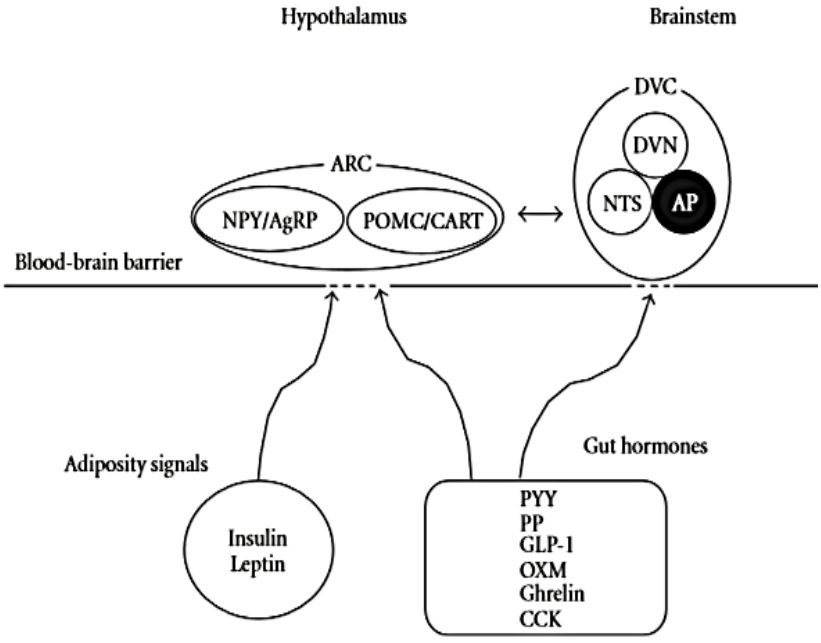

Figure 4. Pancreatic peptide (PP) and other gut hormones interact with complementary receptors, within specific regions of the hypothalamus and brainstem, to modulate appetite. PP interacts with Y4 receptors of the hypothalamic arcuate nucleus (ARC) and neuronal populations within the dorsal vagal complex (DVC) of the brainstem; the dorsal motor nucleus of vagus (DVN), area postrema (AP) and nucleus of the tractus solitarius (NTS) (Suzuki *et al.*, 2011).

Mechanisms of Action

PP, an anorectic peptide, promotes satiety by reducing food intake, the rate of gastric emptying, gall bladder contraction and pancreatic exocrine secretion (Sam *et al.*, 2015).

Appetite Regulation

Interestingly, PP causes both orexigenic and anorexigenic effects according to the route of administration (Katsuura, Asawaka & Inui, 2002). Intracerebroventricular PP infusion in rodents increases appetite and food intake, whilst peripheral administration induces negative energy balance by reducing food intake (Tong *et al.*, 2007). Human subjects of normal weight show a 25% reduction in food intake when infused with PP over 24 hours (Wynne *et al.*, 2005). Asawaka *et al.* (2003) found that intraperitoneal PP administration modifies neuropeptide expression. Hypothalamic anorexigenic peptide, urocortin, gene expression was increased whilst orexigenic peptide, gastric ghrelin, mRNA expression simultaneously decreased (**Fig. 5**). Ghrelin, the only recognised orexigenic peptide (Troke *et al.*, 2014), is elevated in sufferers of Prader-Willi Syndrome (PWS), a disorder distinguished by morbid obesity and hyperphagia (Cummings *et al.*, 2002). PP infusion may improve symptoms of PWS by decreasing ghrelin expression (Asawaka *et al.*, 2003).

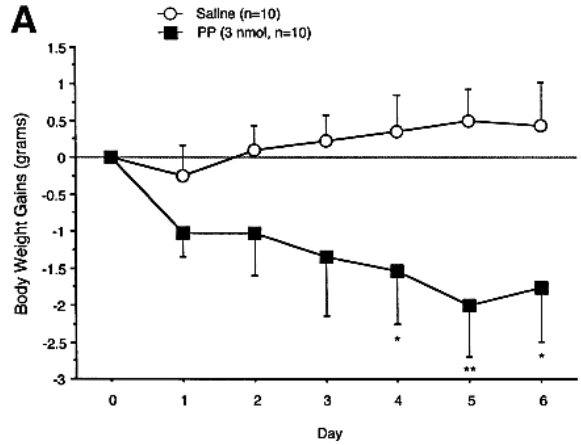

Figure 7. Repeated peripheral pancreatic polypeptide (PP) infusion over a 6-day period significantly decreases body weight gain in rodents. (Asawaka *et al.*, 2003).

Figure 5. Intraperitoneal infusion of pancreatic polypeptide (PP) causes a significant decrease in the expression ghrelin mRNA levels in rodents modulating appetite (Asawaka *et al.*, 2003).

Anxiety

PP administration also significantly decreases corticotrophin-releasing factor (CRF), a hormone activating the hypothalamo-hypophyseal-adrenal axis involved in the pathogenesis of stress-related eating disorders (**Fig. 6**) (Asawaka *et al.*, 2003).

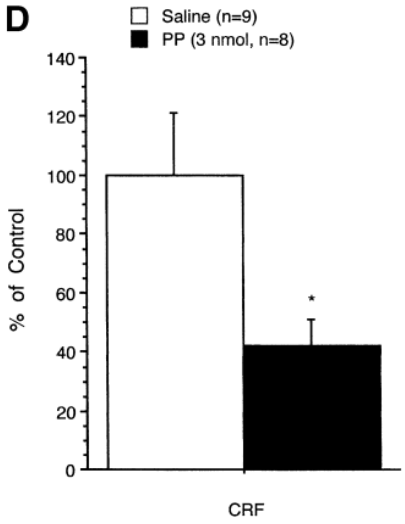

Figure 6. Peripheral administration of pancreatic polypeptide (PP) significantly decreases hypothalamic corticotrophin-releasing factor (CRF) in rodents, potentially moderating anxiety and stress-related eating disorders (Asawaka *et al.*, 2003).

Weight Management

Repeated peripheral PP administration decreases

body weight gain in obese mice (**Fig. 7**). Adiposity and adipocytokines, such as leptin, decrease (Asawaka *et al.*, 2003), potentially preventing hyperleptinaemia and leptin resistance, key contributors to obesity (Sinha & Caro, 1998). PP also improves states of dyslipidemia, reducing plasma free fatty acids, cholesterol and triglyceride levels, as well as ameliorating glycaemic control and insulin resistance (Asakawa *et al.*, 2003).

Sam *et al.* (2015) postulates PP an effective biomarker for ectopic fat deposition as fasting PP concentrations significantly correlate to visceral abdominal and hepatic adiposity. Both pose major risks to cardiometabolic health and contribute to type II diabetes (Tong *et al.*, 2007).

Gastrointestinal Motility

Elevated levels of PP reduce gut motility (Batterham *et al.*, 2003). According to Asawaka *et al.* (2003), transgenic mice, overexpressing PP, show delayed gastric emptying, a classic symptom of anorexia nervosa and cachexia (muscle wasting) in humans. In contrast, obese individuals, expressing low PP, tend to experience increased gastric emptying (Asawaka *et al.*, 2003).

Energy Expenditure

PP influences energy expenditure. Studies show that peripheral PP administration, in obese rodents, increases spontaneous locomotor activity for up to 5 hours (Liu *et al.*, 2008) and increases oxygen consumption for up to 2 hours post-treatment (**Fig. 8**) (Asawaka *et al.*, 2003).

Exocrine and Endocrine Interactions

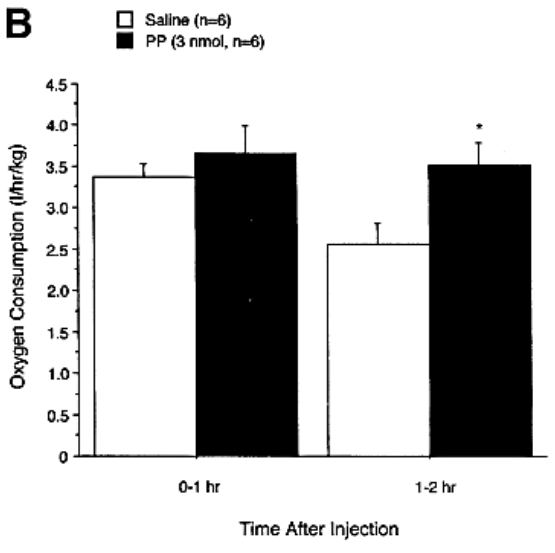

Figure 8. Intraperitoneal pancreatic polypeptide (PP) administration significantly increases oxygen consumption in obese rodents 1-2 hours post-injection, indicating that PP increases energy expenditure (Asawaka *et al.*, 2003).

PP is believed to moderate pancreatic exocrine secretions indirectly through prohibition of insulin activity. Diabetics typically possess elevated PP levels and thus impaired exocrine secretion (Park, Lee & Kwon, 1993). Endocrine secretions, such as delta-cell somatostatin secretion, are also suppressed by PP (Kim *et al.*, 2014).

Pharmacological Therapy

Obesity is one of the most prevalent non-communicable diseases posing a phenomenal challenge to medical professionals globally. Currently, bariatric surgery is the most successful treatment option; post-surgical alterations in gut hormone concentrations have a major influence on sustained weight loss (Troke *et al.*, 2014).

Evidence that PP induces satiety and increases energy expenditure renders it an appealing option for use as an anti-obesity agent; however, pharmacological interventions are unsuccessful long-term (Suzuki *et al.*, 2011). Orally administered peptide pharmaceuticals are rapidly degraded by gastric enzymes (Troke *et al.*, 2014), easily excreted and have a limited shelf-life

(Bellmann-Sickert *et al*., 2011). PP analogues, designed with pharmacokinetic modifications such as fatty acid or polyethylene glycol acylation (Bellmann-Sickert *et al*., 2011), enhance efficacy in modulation of appetite and extend PPs short half-life of 7 minutes, eliminating necessity for prolonged infusion (Troke *et al*., 2014).

It must be appreciated that gut-hormone therapies may not suffice as a single solution for obesity treatment (Chaudhri *et al*., 2008). The gut-brain axis does not operate in isolation but in conjunction with complex cortical centers and reward-related corticolimbic pathways of the brain, responsible for hedonistic control (Suzuki *et al*., 2011). Social, environmental and emotional factors also influence food intake in humans, overriding homeostatic requirements. Furthermore, as most research discussed utilises rodent models, one may question whether PP exerts identical physiological effects in humans.

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