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Protein digestion and energy homeostasis: How generated peptides may impact intestinal hormones?

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ABSTRACT

The aim of the present study is to investigate how peptides released by gastrointestinal (GI) digestion of one dietary protein can interact with regulating processes of food intake. An in vitro GI digestion of bovine haemoglobin was carried out and the bioactivity of the digests on CCK and GLP-1 secretion and dipeptidyl peptidase IV (DPP-IV) activity was measured. Intestinal digests exhibited the most potent action on gut hormone release and DPP-IV activity inhibition. They also had the ability to promote hormone gene expression. As a conclusion, two fractions from final intestinal digest led to the greatest GLP-1 secretion increase and DPP-IV activity inhibition. These findings could be very useful in obesity and T2D management research.

1. Introduction

Food intake regulation strongly relies on the gut-brain axis and numerous studies have pointed out the significant role played by gut-derived hormones released from enteroendocrine cells (EECs) in response to food digestion (Cummings & Overduin, 2007). The gut produces a variety of hormones from the small intestine (cholecystokinin) and from the lower small intestine (glucagon-like peptide 1, Peptide YY, oxyntomodulin) released from the enteroendocrine cells (EECs) scattered among epithelial cells and representing less than 1% of the entire gut epithelial population. These hormones are involved in appetite regulation as short-term peripheral satiety signals: they promote satiety while decreasing appetite by activating different signalization pathways, (Moran & Dailey, 2009; Morton, Cummings, Baskin, Barsh, & Schwartz, 2006; Wren & Bloom, 2007). Facing the worldwide issue of obesity, the anorexigenic properties of gut hormones make them good candidates for new obesity treatments (Perry & Wang, 2012; Troke, Tan, & Bloom, 2014). Proteins are nowadays well-known to be the most satiating macronutrient compared with carbohydrates and fat. Up to date, known mechanisms that contribute to protein-induced satiety involve gut-derived hormone increase, mainly cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1), thermogenesis and gluconeogenesis stimulation (Veldhorst et al., 2008; Westerterp-Plantenga, 2008). After ingestion, proteins are first partially hydrolysed by pepsin in the stomach. Protein digestion continues in the small intestine achieved by a cocktail of proteases (trypsin, chymotrypsin, carboxypeptidases) and is extended by brush border membrane peptidases in the microvilli of epithelial cells. Generated peptides are able to interact with the EECs and to stimulate CCK and GLP-1 release. Protein hydrolysates from soy, pea, potato, milk (Foltz et al., 2008), fish (Cudennec, Fouchereau-Peron, Ferry, Duclos, & Ravallec, 2012) or meat (Sufian et al., 2006) exhibited CCK-releasing properties. However the correlation between GLP-1 plasma levels and protein intake still remains controversial (Diakogiannaki, Gribble, & Reimann, 2012). For both peptide hormones precise cellular and molecular mechanisms of production and secretion regulation are still under investigation. In addition to its role as peripheral satiation signal and in appetite regulation, GLP-1 is also regarded as a prime incretin hormone, i.e. it potently stimulates glucose-dependent insulin secretion. However, circulating GLP-1 levels increase after meal intake but rapidly decrease due to enzymatic inactivation by dipeptidyl peptidase IV (DDP-IV) and intact biologically active GLP-1 represents only 10–20% of total plasma GLP-1. Therefore, inhibiting DPP-IV activity has become one of the strategies for type 2 diabetes treatments (D. J. Drucker, 2006; Godinho et al., 2015) and many molecules DPP-IV inhibitors (such as gliptins) have been developed and commercialized to increase postprandial intact GLP-1 plasma levels (D. J. Drucker & Nauck, 2006). DPP-IV is a serine protease expressed in both membrane and soluble forms in a wide

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variety of cells, particularly on epithelial tissues such as liver, kidney and intestine (Lorey et al., 2003). This enzyme strictly removes dipeptides from the N terminus of oligopeptides and preferentially cleaves behind penultimate N-terminal Proline or Alanine residues. DPP-IV is also involved in various regulatory processes other than incretin hormones (GLP-1 and GIP) inactivation and is important in neuropeptides metabolism or chemokines attraction and immunity (Engel et al., 2006). Over the past few years, attention has risen towards dietary proteins as precursors of DPP-IV inhibitors. Though, Inhibitory peptides need to be released by in vitro hydrolysis or in vivo digestion to exert their bioactivity. Numerous protein hydrolysates and peptide sequences from both animal and plant protein sources have already been reported as DPP-IV activity inhibitors, mainly from whey proteins (A. B. Nongonierma & FitzGerald, 2013; Tulipano, Sibilia, Caroli, & Cocchi, 2011) in particular β-lactoglobulin (Silveira, Martínez-Maqueda, Recio, & Hernández-Ledesma, 2013) and α-lactalbumine (Lacroix & Li-Chan, 2014) but also defatted rice bran (Hatanaka et al., 2012) or tuna cooking juice (Huang, Jao, Ho, & Hsu, 2012). CCK and GLP-1 secretion studies and DPP-IV inhibition investigations often use in vitro partially or extensively hydrolysed proteins. However, how bioactive peptides could be released from the parent protein during gastrointestinal (GI) digestion is not well understood yet.

The aim of the present study was to investigate the effects of bovine haemoglobin digests, as a dietary animal protein model, produced by an in vitro GI digestion model on CCK and GLP-1 secretion regulation and DPP-IV activity inhibition. Secretion regulation studies were performed at protein and mRNA levels in the widely established STC-1 cells as small intestine EECs in vitro model and DPP-IV inhibitory activity of digests was assessed by in vitro tests.

2. Materials and methods

2.1. Materials

Bovine haemoglobin, porcine pepsin (EC 3.4.23.1, from porcine gastric mucosa, >250units.mg^{-1} solid), pancreatin from porcine pancreas (4xUSP specifications, EC number 232–468-9), Gly-Pro-p-nitroanilide (H-Gly-Pro-pNA/HCl), Dipeptidyl Peptidase IV (DPP-IV from porcine kidney, EC 3.4.14.5, ≥10 units per mg protein) and all other reagents were purchased from Sigma-Aldrich. Active Glucagon-Like Peptide RIA kit (Cat.# GLP1A-35HK) was purchased from EMD Millipore (Merck KGaA, Darmstadt, Germany) and RIA kit (GASK-PR) for the determination of CCK was purchased from Cisbio Bioassays (Codolet, France).

2.2. In vitro gastrointestinal digestion of bovine haemoglobin

A static mono compartmental digestion protocol was set up in order to reproduce the human gastrointestinal (GI) environment as closely as possible, as described by Versantvoort et al. The three first steps of digestion were simulated (mouth, stomach and small intestine) and three fluids were prepared to mimic the physiological conditions of each step (Versantvoort, Oomen, Van de Kamp, Rompelberg, & Sips, 2005). The composition of each fluid is described in Table 1 and pH solutions were adjusted to physiologically relevant values using 5 M NaOH and 5 M HCl solutions. The whole digestion process was performed in a 200 mL reactor controlled at 37 °C under constant stirring with a magnetic stirrer over 240 min. Sampling of 4 mL was performed every 30 min after the saliva step. Digests were named according to their sampling time (from 30 to 120 min) and digestion step (gastric or intestinal). Two grams of haemoglobin powder was added to the reactor and solubilized in 16 mL salivary fluids at pH 6.8 at a final concentration of 125 g.L^{-1} dry matter. 24 mL of gastric fluids containing pepsin at a E:S ratio of 1:40 (*w*/*w*) were added after saliva sampling and pH solution was adjusted (2.5–3.0). After 2 h, 36 mL of intestinal fluids containing pancreatin at a E:S ratio of 1:50 (*w*/*w*) and 4 mL of 1 M $NaHCO_3$ were added to the batch, pH solution was adjusted to 7 and intestinal digestion was carried out again over two hours. Final digest concentration reached 13.89 g.L^{-1} dry matter. Once heated at 95 °C for10 min to assure enzyme denaturation, all samples were centrifuged at 13,400 *g* for 10 min. Supernatants were collected, filtered through 0.22 μm filters and frozen for further analysis. Prior to chromatography separation and DPP-IV activity assay, haem was removed from digests by precipitation (except for saliva sample) by adding 40 μL of HCl 1 M per mL of hydrolysate and samples were then centrifuged at 13,400 *g* for 5 min. Supernatants were collected and stored at −20 °C before use.

2.3. Size exclusion chromatography (SEC)

Haemoglobin 120 min-intestinal digest was separated using a HiLoad 16/600 Superdex 30 prep grade column (GE Healthcare, Uppsala, Sweden) on an AktaPurifier (GE Healthcare, Uppsala, Sweden). Separation was carried out under isocratic elution at a flow rate of 1 mL.min^{-1} with a 30% acetonitrile, 0.1% TFA solvent. 2 mL of the decolorized supernatant (prepared as described in 2.2) at a concentration of 1.4% (*w*/*v*) was injected and absorbance was monitored at 214 nm. Four fractions were collected manually following the sampling plan on Fig. 2. They were dried and stored at −20 °C for further analysis. The column was calibrated using standard peptides (Albumin, 60 kDa; Cytochrome C, 12,400 Da; Aprotinin, 6500 Da; Vitamin B12, 1355 Da; Glutathione 307 Da). To perform the molecular weight profiles of the hydrolysates, the relationship between Log of molecular weight standard peptides and elution volume was used.

2.4. LC–MS analysis of digest fractions

LC–MS analysis was performed on a UFLC-XR device (Shimadzu, Kyoto, Japan) coupled to a QTRAP® 5500 MS/MS hybrid system triple quadrupole/linear ion trap mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with a Turbo VTM ion source. Instrument control, data acquisition and processing were performed using the associated Analyst 1.5.2 software. The RPLC separation was carried out on a Kromasil C18 column (100 × 2.1 mm, 3.5 μm) with guard cartridge from AIT-France. Fractions were solubilized at 0.4% (*w*/*v*) using water, 0.1% formic acid. The injection volume was 20 μL. Elution was performed at a flow rate of 200 μL/min with water-formic acid 0.1% as

Table 1
Chemical composition, protease concentrations and pH used for the different fluids of the in vitro simulated gastrointestinal digestion.

	Saliva	Gastric Juice	Duodenal Juice	Bile juice
Chemical composition	KCl (6 mM)	KCl (5.5 mM)	KCl (3.8 mM)	KCl (2.5 mM)
	KSCN (1 mM)	NaH_2PO_4 (1 mM)	KH_2PO_4 (0.3 mM)	NaCl (60 mM)
	NaH_2PO_4 (3.7 mM)	NH_4Cl (2.8 mM)	NaCl (60 mM)	$NaHCO_3$ (3.4 mM)
	Na_2SO4 (2 mM)	NaCl (2.4 mM)	$NaHCO_3$ (3.4 mM)	HCl (10 mM)
	NaCl (2.5 mM)	HCl (78 mM)	HCl (10 mM)	$CO(NH_2)_2$ (80 μM)
	$NaHCO_3$ (10 mM)	$CaCl_2$ (1.8 mM)	$MgCl_2$ (0.1 mM)	
	$CO(NH_2)_2$ (1,7 mM)	$CO(NH_2)_2$ (80 μM)	$CO(NH_2)_2$ (80 μM)	
Proteases		Pepsin 1/40 (*w*/*w*)	Pancreatin 1/50 (*w*/*w*)	
pH	6.8 ± 0.2	1.3 ± 0.2	8.1 ± 0.2	8.2 ± 0.2

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