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Important species differences regarding lymph contribution to gut hormone responses

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ABSTRACT

Introduction: GLP-1 is secreted from the gut upon nutrient intake and stimulates insulin secretion. The lymph draining the intestine may transport high levels of GLP-1 to the systemic circulation before it is metabolized by DPP-4. The aims of this study were to investigate to what extent the lymphatic system might contribute to the final level(s) of systemic circulating intact GLP-1 and, in addition, whether secretory profiles in intestinal lymph might reflect lamina propria levels of GLP-1 i.e. before capillary uptake and degradation by endothelial dipeptidyl peptidase-4 (DPP-4).
Method: 7 pigs of the YDL-strain were catheterized in the portal vein, carotid artery and cisterna chyli (lymph). Neuromedin C (NC) was infused through an ear vein catheter, before and after injection of a selective DPP-4 inhibitor (vildagliptin). Total and intact GLP-1 levels were measured throughout the 150 min experiments using specific sandwich ELISAs. DPP-4 activity was measured spectrophotometrically.
Results: Concentrations of both total and intact GLP-1 were markedly lower in lymph compared to plasma samples, and did not increase significantly in response to stimulation with NC in the absence/presence of vildagliptin. In contrast, total and intact GLP-1 levels increased significantly in the portal vein and carotid artery. DPP-4 activity was lower in lymph than plasma, and was reduced further by vildagliptin.
Conclusion: Our observations indicate that the lymphatic system does not transport high levels of intact GLP-1 to the systemic circulation, and that GLP-1 levels in cisternal lymph do not reflect the hormone levels in the intestinal lamina propria.

Introduction

Glucagon-like peptide-1 (GLP-1), secreted from intestinal L-cells, regulates glucose levels by potentiating glucose-stimulated insulin secretion (the incretin effect), supressing glucagon secretion, and inhibiting appetite [4,14,19]. Because of these features, GLP-1 receptor agonists are currently used as treatment in patients with type 2 diabetes (T2DM). After secretion, intact (active) GLP-1 (7–36 NH_2) diffuses to the lamina propria, and enters the local capillaries, where it is rapidly degraded by dipeptidyl peptidase 4 (DPP-4) located on the capillary endothelium to its metabolite (9–36 NH_2), This process is highly efficient, with only 20–30% reaching the portal vein in the intact form, and less than 15% circulating as the intact peptide [5]; indeed, it has been calculated that only 8% of newly secreted GLP-1 actually reaches peripheral targets in the intact form [13]. Circulating GLP-1 levels are, therefore, low, which complicates measurement and assessment of potential GLP-1 stimulating drugs (secretagogues). However, it has also been suggested that GLP-1 might enter the lymphatic capillaries (lacteals) together with other nutrients e.g. chylomicrons [17], with the lymph system acting as a conduit for transporting high levels of GLP-1 from the intestinal mucosa to the systemic circulation [17], Moreover, since DPP-4 activity was reported to be less in lymph compared to plasma [17], measurement of intact GLP-1 in lymph has also been suggested as a novel model for studying the effects of GLP-1 secretagogues [15].

The primary aim of this study was to investigate whether the lymphatic system in anaesthetized pigs carries high levels of intact GLP-1 to the systemic circulation. In addition, we hypothesized that

Abbreviations: DPP-4, dipeptidyl peptidase 4; GLP-1, glucagon-like peptide-1; NC, bombesin/neuromedin C.

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GLP-1 levels in lymph might reflect its concentrations in the interstitial intestinal fluid of the lamina propria i.e. its local site of action, where it is been proposed to interact with sensory afferents before it reaches the capillaries and is degraded by DPP-4 [10].

Materials and methods

The animal studies were conducted in accordance with international guidelines (National Institutes of Health publication no. 85-23, revised 1985, and Danish legislation governing animal experimentation, 1987), and were carried out after permission had been granted by the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

Animal and surgical experiments

7 young pigs of the YDL-strain weighing 34.7 ± 3.4 kg (mean ± SD) were fasted overnight, but had free access to drinking water. After premedication with ketamine (10 mg/kg, Ketalar, Parke-Davis, Morris Plains, NJ) and induction with pentobarbital (Mebumal®, Nycomed DAK, Copenhagen, Denmark), the animals were anaesthetized with iv α-chloralose (50 mg/kg, Merck & Co, Darmstadt, Germany) and ventilated with intermittent positive pressure using N_2O/O_2 (AGA, Copenhagen, Denmark). Catheters were placed in the left ear vein for peptide administration and the right ear vein for DPP-4 inhibitor administration (vildagliptin; kindly provided by Novartis Institutes Biomedical Research, Inc., Cambridge, USA). Catheters were placed in the right carotid artery and in the portal vein as previously described [6]. Additionally, a non-obstructing catheter was fixed to the walls of the cisterna chyli by means of a purse-string suture for lymph sampling. The lymph flow during the entire experiment was sufficient for sample collection. After surgical preparation, the animals were heparinised and left undisturbed for 30 min.

Experimental protocol

In order to stimulate the release of endogenous GLP-1, the animals received two intravenous boluses of neuromedin C [120 nmol] [11,13] (cat#3120, Bachem, Bubendorf, Switzerland) via the left ear vein catheter, one before and one after vildagliptin (1 mg/kg) administration. Simultaneous blood (3.5 ml) and lymph (1 ml) samples were collected over 150 min from the carotid artery, portal vein and cisterna chyli, respectively, into chilled tubes containing EDTA (7.2 nmol/l, final concentration), aprotinin (500 KIE/ml blood) and the DPP-4 inhibitor, valine-pyrrolidide (0.01 mmol/l, final concentration; a gift from Novo Nordiak, Bagsvaerd, Denmark). The flow of lymph in the cisterna chyli was sufficient to allow sampling by cautious suction on the catheter during the entire sampling time. For DPP-4 activity measurements, 0.5 ml (of each sample) was dispensed into tubes containing only EDTA. The tubes were kept on ice until centrifugation at 4 °C, and the supernatant stored at −20 °C until analysis. The blood volume taken did not exceed 10% of the total blood volume, but fluid loss was replaced after each blood sample with corresponding volumes of saline [8]. Both heart rate and blood pressure were monitored continuously and remained stable throughout the experiment.

Peptide analysis

Total GLP-1 levels were measured using Millipore Total GLP-1 ELISA kit (cat# EZGLP1T-36K, Millipore, Billerica, MA, USA) as described previously [2]. Intact GLP-1 levels were measured using an in-house 2-site sandwich ELISA for GLP-1 as described previously [18]. DPP-4 activity was assessed kinetically by standard procedures, using H-Gly-Pro-p-nitroaniline as substrate [16], with minor modifications.

Statistical methods

Total GLP-1 and intact GLP-1 responses were evaluated by AUC (total AUC: whole experiment, incremental AUC: stimulation period with NC) calculated using the trapezoidal rule, tested by paired *t*-test, and two-way ANOVA for repeated measurement followed by Bonferroni post hoc analysis as appropriate. DPP-4 activity was evaluated using a paired *t*-test. All data are presented as mean ± standard deviation (SD), *P*-values of <0.05 were considered significant. Statistical analysis was carried out using Graphpad Prism version 6.00 for Windows, GraphPad Software, La Jolla, California, USA.

Results

Total GLP-1 levels (see Fig. 1A) were higher ($P < 0.05$) in the portal vein compared to the carotid artery, and each was markedly ($P < 0.05$) higher compared to lymph (4680 ± 2381 pmol/l min, 3067 ± 1251 pmol/l min and 373 ±226 pmol/l min AUC_{total} respectively). Intact GLP-1 levels at all three sites were lower than total GLP-1 levels (see Fig. 1B), but the pattern was similar, with highest concentrations in the portal vein ($P < 0.05$), and levels in both vascular vessels being greater ($P < 0.05$) compared to lymph (853 ± 277 pmol/l min, 689 ± 185 pmol/l min and 134 ± 99 pmol/l min AUC_{total} respectively).

Total GLP-1 levels measured during NC stimulation alone ($AUC_{incremental}$) increased significantly ($P < 0.05$) in plasma but not in lymph ($P = 0.33$). The inclusion of the DPP-4 inhibitor, vildagliptin had no effect on total GLP-1 concentrations during NC stimulation (being similar without and with vildagliptin, portal vein: 216/236 pmol/l min; carotid artery: 162/178 pmol/l min; lymph: 16/17 pmol/l min). In contrast, intact GLP-1 (see Fig. 1B) levels in both portal vein and carotid artery ($P < 0.05$), but not in lymph ($P = 0.17$), increased significantly in response to NC when DPP-4 was inhibited.

DPP-4 activity (see Fig. 1C) was significantly higher (1.5 fold, $P < 0.05$) in plasma (carotid artery) compared to lymph. Vildagliptin significantly ($P < 0.05$) reduced DPP-4 activity in both plasma and lymph (by 80 ± 6% and 81 ± 4%, respectively; values not corrected for dilution in the assay), but absolute activity still remained higher in plasma.

Discussion

In the current study, we investigated whether the lymphatic system transports high levels of intact GLP-1 from the intestine (lamina propria) to the systemic circulation, using anaesthetized pigs. We used the pig as a model because of its physiological similarities to human, in terms of digestive and associated metabolic processes [12], and since it allowed us to collect multiple larger (volume) samples compared to rodents. By estimating both intact and total GLP-1 in lymph and in plasma from portal vein and carotid artery simultaneously, we were able to identify the systemic, mesenteric and lymph GLP-1 ratios under basal conditions and during stimulation with NC (a powerful GLP-1 secretagogue), with or without simultaneous inhibition of the DPP-4 enzyme. This allowed us to estimate the sites of important DPP-4-mediated inactivation of the hormone. In particular we hypothesized that DPP-4-mediated degradation would be small in intestinal lymph, because GLP-1 is not expected to come into contact with DPP-4 before it enters the capillaries of the gut (and again in the liver).