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Separation of emetic and anorexic responses of exendin-4, a GLP-1 receptor agonist in *Suncus murinus* (house musk shrew)

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ABSTRACT

The use of glucagon-like peptide-1 (7–36) amide (GLP-1) receptor agonists for the treatment of type 2 diabetes mellitus is commonly associated with nausea and vomiting. Therefore, the present studies investigated the potential of GLP-1 receptor ligands to modulate emesis and feeding in *Suncus murinus*. Exendin-4, a selective GLP-1 receptor agonist, was administered subcutaneously (1–30 nmol/kg) or intracerebroventricularly (0.03–3 nmol) after 12-h of fasting. In other studies, animals were pretreated with the GLP-1 receptor antagonist, exendin (9–39), or saline (5 μl) 15 min prior to exendin-4 (3 nmol, i.c.v.). Behaviour of animals and food and water intake were then recorded for 1–2 h; c-Fos expression was also assessed in the brains of animals in the i.c.v. studies. The subcutaneous administration of exendin-4 reduced food and water intake ($p < 0.001$) and induced emesis in 40% of animals ($p > 0.05$). The intracerebroventricular administration of exendin-4 also prevented feeding, and induced emesis ($p < 0.01$). In these studies, exendin (9–39) (30 nmol, i.c.v.) antagonised emesis induced by exendin-4 and the increased c-Fos expressions in the brainstem and hypothalamus ($p < 0.05$), but it was ineffective in reversing the exendin-4-induced inhibition of food and water intake ($p > 0.05$). These data suggest that exendin-4 exerts its emetic effects in the brainstem and/or hypothalamus via GLP-1 receptors. The action of exendin-4 to suppress feeding may involve non-classical GLP-1 receptors or other mechanisms.

1. Introduction

The therapeutic options for the treatment of type 2 diabetes mellitus (T2DM) have increased dramatically in the last decade. An emerging novel therapeutic class of anti-diabetic drug is glucagon-like peptide-1 (7–36) amide (GLP-1) receptor agonists. GLP-1 is a potent blood glucose-lowering hormone produced by intestinal L cells via tissue specific post-translational processing of the pro-glucagon gene (Orskov et al., 1989). In response to nutrient ingestion (Kreymann et al., 1987), GLP-1 is secreted into the circulation to potentiate glucose-stimulated insulin secretion (Kreymann et al., 1987); vagovagal reflexes via the dorsal motor nucleus also play a role (Mussa and Verberne, 2013). GLP-1 also reduces food intake and enhances satiety in rats and humans, both lean and obese (Flint et al., 1998; Naslund et al., 1998; Turton et al., 1996).

Exenatide was the first GLP-1 receptor agonist approved as an adjunct therapy to improve glycaemic control in patients with T2DM. Unfortunately, its use was associated with adverse effects on the gastrointestinal tract. A 30-week, randomised, double-blinded, parallel, placebo-controlled study revealed that nausea (41% vs. 8%) and vomiting (18% vs. 4%) were significantly higher with exenatide than placebo (Buse et al., 2011). In healthy volunteers, the administration of exenatide (10 μg) reduced significantly appetite (43% vs. 10%) and induced nausea (63% vs. 20%), and vomiting (18% vs. 0%) compared with placebo (Pinelli et al., 2011). It was proposed that the adverse effects of nausea and vomiting are associated with the plasma concentration of exenatide. Indeed, a careful dose-escalation of exenatide is associated with a lower incidence of nausea and vomiting in patients with T2DM (Deyoung et al., 2011).

Previous pre-clinical studies suggest that GLP-1 receptors may have an important role in the emetic pathways. A discrete set of hindbrain neurons in the central nervous system (CNS) produces GLP-1 and these neurons send projections to multiple regions of the brainstem, hypothalamus, and the forebrain limbic system (Jin et al., 1988; Larsen et al., 1997), where GLP-1 receptor expressing

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cells are coexpressed (Goke et al., 1995; Merchenthaler et al., 1999). Indeed, intracerebral third ventricular (i3vt) administration of GLP-1 induces conditioned taste aversion (CTA) (Thiele et al., 1997) and increases the plasma levels of arginine vasopressin in rats (Larsen et al., 1997). Furthermore, GLP-1 and lithium chloride (a treatment also producing CTA) induce a similar pattern of c-Fos expression in the CNS, including neurons in the hypothalamus, central nucleus of the amygdala (CeA), and brainstem (Rinaman, 1999a,b). These findings suggest that GLP-1 may be a candidate for mediating the neuroendocrine and behavioural effects of nauseagenic treatments. A more recent study has been conducted using exendin-4 and the longer acting GLP-1 agonist, liraglutide, in rodent models of CTA and pica (ingestion of kaolin as a putative index of nausea). Following peripheral administration, both agents induced CTA but only exendin-4 induced reliable pica over a 12-day period; the effect on food consumption was more complex in terms of a separation of potency between the paradigms. It was also shown that the effect of exendin-4 to induce pica and decrease food intake was independent of the peripheral vagi (Kanoski et al., 2012).

Experiments using CTA and pica paradigms have been considered useful to provide information relative to mechanisms of nausea and/or emesis in non-vomiting species, but they both rely on indices generated by changes of ingestive behaviour. Information gained from studying pathways controlling ingestion, may be different from those controlling gastric expulsion (emesis and retching). To date, most of the preclinical studies on GLP-1 function have used laboratory animals incapable of emesis. *Suncus murinus* (house musk shrew) is commonly used in emesis research where the distribution of monoaminergic neurones throughout the brain is well documented, and where the components of the dorsal vagal complex have been well defined (Holmes et al., 2009; Karasawa et al., 1991; Ueno et al., 1987; Won et al., 1998). In this species, we have shown that the GLP-1 receptor agonist exendin-4 potently contracts the isolated ileum of *S. murinus* (pEC_{50} 8.4 ± 0.4) and exendin (9–39) is a selective reversible antagonist (PK_B value of 9.7) (Chan et al., 2007). We have previously also shown that exendin-4 reduces blood glucose in anaesthetised *S. murinus* following a peripheral and i.c.v. administration; we also provided evidence for an involvement of GLP-1 receptors in the ventromedial hypothalamus (Chan et al., 2011).

In the present studies, therefore, we used *S. murinus* to investigate if exendin-4 induces emesis at around doses previously shown to affect blood glucose levels, and if it also affects food and water intake and locomotor activity in conscious animals. In previous studies using ferrets which also have a capacity to vomit, it was shown that brainstem GLP-1 pathways connecting with the forebrain are activated by treatments causing CTA. Therefore, we also examined if similar pathways are activated by exendin-4 using c-Fos immunohistochemistry, which has been used successfully to identify brainstem areas involved in emesis control in the ferret (Reynolds et al., 1991; Zaman et al., 2000). Finally, we investigated the specificity of action of behaviours and c-Fos changes using the GLP-1 receptor antagonist, exendin (9–39) (Schirra et al., 1997).

2. Materials and methods

2.1. Animals

Female *S. murinus* (30–40 g) were obtained from the Chinese University of Hong Kong and housed in a temperature-controlled room at 24 ± 1 °C under artificial lighting, with lights on between 0600 and 1800 h. Humidity was maintained at 50 ± 5%. Water and dry cat chow pellets (Feline Diet 5003, PMI® Feeds, St. Louis, USA) were given *ad libitum*. Some groups of animals underwent stereotaxic surgery for the placement of guide cannulae. All experiments were conducted under licence from the Government of Hong Kong SAR and with permission from the Animal Experimentation Ethics Committee, The Chinese University of Hong Kong.

2.2. Stereotaxic surgery

Animals were anaesthetised with sodium pentobarbitone (40 mg/kg, i.p.) and then stereotaxically implanted with a 23-gauge stainless steel guide cannulae into the right lateral ventricle at 0.9 mm lateral to the midline, 8.2 mm posterior to lambda, and 1.2 mm below the dura (Rudd and Wai, 2001). After cannulation, the animals were administered buprenorphine (0.05 mg/kg, s.c.) as a postoperative analgesic and then individually housed and allowed a 5-day recovery before the *commencement of the experiment.* During drug administration, the guide cannula was fitted with a 30-gauge stainless steel injection needle that extended 0.5 mm beyond the tip of the guide cannula. At the end of the experiments, 5 μl of methylene blue dye was injected i.c.v. following termination of the animals with pentobarbitone (80 mg/kg, i.p.), and the brains removed to confirm the site of injection. Only those animals having blue staining in the lateral and fourth ventricles were included in the analysis.

2.3. Administration of drugs

One day prior to experimentation, animals were transferred to the observation room with controlled lighting (15 ± 2 Lux) and habituated to clear Perplex observation chambers (21 × 14 × 13 cm^3). The animals were food deprived 12 h prior to administration of drugs; water was given *ad libitum* unless otherwise stated. In some experiments, animals were injected subcutaneously (s.c.) with exendin-4 (1, 10 and 30 nmol/kg, 0.2 ml/kg) or saline (0.2 ml/kg) and observed for 120 min. In animals that had been prepared stereotaxically, intracerebroventricular injection of exendin-4 (0.3, 1 and 3 nmol) or saline (5 μl) was given and then observed for 60 min. In other experiments, animals were injected with exendin-4 (3 nmol, i.c.v.) or saline (5 μl) 15 min after the administration of exendin (9–39) (30 nmol, i.c.v.) or saline (5 μl). In the latter experiments, the animals were anaesthetised 60 min post exendin-4 administration, and the brains were removed and processed for c-Fos immunohistochemistry (see below). In all the experiments, except those involving c-Fos immunohistochemistry, cat chow pellets and water were presented 15 min after the administration of exendin-4 or its vehicle (i.e. saline). Episodes of emesis were characterised by rhythmic abdominal contractions that were associated with either oral expulsion of solid or liquid materials from the gastrointestinal tract (i.e. vomiting) or without the passage of materials (i.e. retching movements). Two consecutive episodes of retching and/or vomiting were considered separate when an animal changed its location in the observation chamber or when the interval between retches and/or vomits exceeded 2 s. Changes in locomotor activity were measured captured by a closed circuit camera (Panasonic, WV-PC240, China) connected to an EthoVision Colour Pro system (Version 2.3; Noldus Information Technology, Costerweg, Netherlands) running on a personal computer (Lau et al., 2005). Food and water intake was measured at the end of the observation periods.

2.4. c-Fos immunohistochemistry

Animals were anaesthetised with sodium pentobarbitone (40 mg/kg, i.p.) and perfused intracardially with ice-cold saline (120 ml) followed by 4% paraformaldehyde in phosphate-buffered saline (PBS; 100 ml). The brains were then removed and postfixed in 4% paraformaldehyde overnight at 4 °C. Frozen tissues were then sectioned at 40 μm in the coronal plane using a freezing microtome and incubated at room temperature for 1 h in 0.01% H_2O_2. The free floating sections were blocked with 1.5% normal goat serum containing 0.3% Triton X-100 in PBS (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, USA) for 1 h. Sections were then incubated with rabbit anti-c-Fos antibody (1:10,000, Ab5, Oncogene Research Products, Cambridge, USA) without washing for 48 h at 4 °C. The sections were subsequently washed and incubated with secondary goat-anti-rabbit antibody (1:200; Vector Laboratories) for 1 h, followed by Vectastain *avidin–biotin complex reagent* for 1 h (1:100; Vectastain Elite ABC kit, Vector Laboratories, Burlingame, USA). c-Fos expression was visualised using a commercially available peroxidase substrate (Vector® VIP kit, Vector Laboratories, Burlingame, USA).

2.5. Quantification of c-Fos immunoreactive cells

The number of Fos-like-immunoreactive nuclei was counted manually with the aid of a Zeiss Axioskop 2 plus microscope (Carl Zeiss Inc., Thornwood, USA) equipped with a Zeiss Axiocam 2 camera. To quantify c-Fos expression in hypothalamic nuclei, three representative sections were selected in accordance with the stereotaxic atlas constructed in our laboratory, based on the stereotaxic atlas of the mouse brain (Frankin and Paxinos, 2008). Specifically, the anterior–posterior coordinates (measured from lambda) of sections in which c-Fos were counted were +5.32, +5.44 and +5.56 for the ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH), peripheral lateral hypothalamus (PLH) and arcuate nucleus (Arc); +5.92, +6.04 and +6.28 for the central nucleus of the amygdala (CeA) and paraventricular hypothalamus (PVH); −0.38, −0.26, −0.14 and −0.02 for the area postrema (AP) and nucleus tractus solitarius (NTS). For the forebrain areas, a grid of 200 × 200 μm^2 was superimposed on the centre of each nucleus; c-Fos positive cells on both sides of each section were manually counted and observed at ×10 magnification. For the brainstem areas, a grid of 100 × 100 μm^2 was superimposed on the centre of each nucleus; c-Fos-positive cells on both sides of each section were