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Insights into the central pathways involved in the emetic and behavioural responses to exendin-4 in the ferret

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ABSTRACT

Background: GLP-1 receptor agonists are utilised for the treatment of Type-2 diabetes but can be associated with undesirable effects of nausea and vomiting.
Objectives: To investigate the role of GLP-1 receptors in mechanisms of emesis, behaviours indicative of nausea (BIN) and food intake in the ferret.
Results: Exendin-4 (10 and 30 nmol, i.c.v.) induced emesis, inhibited food intake, and increased the frequency of BIN. Increases in c-Fos in the brainstem, midbrain and forebrain occurred in animals exhibiting emesis; no activation of the brainstem occurred in animals not vomiting. Exendin-4 (10 nmol, i.c.v.) when preceded by i.c.v. saline (15 μl), was not emetic but induced BIN and inhibited food intake; exendin (9–39) (100 nmol) reduced BIN only. c-Fos showed that consistent with the absence of emesis in saline/exendin-4 treated animals there was no increase in c-Fos in the brainstem, but it increased in midbrain and forebrain nuclei. Excepting the amygdala, exendin (9–39) was without efffect on the increases in c-Fos. Analysis of c-Fos data showed a positive linear relationship between midbrain and forebrain areas irrespective of the occurrence of emesis induced by exendin-4. In contrast, brainstem and midbrain c-Fos levels were positively correlated, but only in animals with emesis.
Conclusions: The brainstem is critical for exendin-4-induced emesis but suppression of food intake and BIN involves more rostral brain sites. Exendin-4-induced BIN and c-Fos activation of the amygdala are sensitive to exendin (9–39), whereas the suppression of food intake is not implicating separate control mechanisms for emesis and BIN.

1. Introduction

Peptide hormones released from the digestive tract can act on local target tissues including smooth muscle, interstitial cells of Cajal (ICC), epithelial cells and neurones (e.g. enteric nervous system, vagal afferents) (Blair et al., 2014; Brookes et al., 2013; Furness, 2012; Williams et al., 2016). The action on vagal afferent terminals either directly via receptors or secondary to an action of another process (e.g. disruption of motility, secretion from enterochromaffin cells) can also modulate the central nervous system (Brookes et al., 2013; Dockray, 2014; Sanger et al., 2013). Peptide hormones may also act on the brain within the circumventricular organs located in the third (e.g. organum vasculosum lamina terminae, subfornical organ (OVLT), bed nucleus of the stria terminalis (BNST)) or fourth ventricles (area postrema; AP), as the blood brain barrier (BBB) is relatively permeable in these regions (Borison, 1989; Johnson and Gross, 1993; Kash et al., 2015). Peptides can also act on the brain if transported across the BBB, or if the BBB becomes permeabilised by disease (e.g. local inflammation, cerebral oedema) (Banks, 2006). Substances in the cerebrospinal fluid CSF can influence brain function and autonomic outflow via access to the circumventricular organs where the CSF-brain barrier is similarly relatively permeable (Leslie, 1986).

There has been an increasing interest in the central effects of gastrointestinal peptides because of their involvement in the control of food intake and in the sensations of hunger and satiety. Glucagon like peptide (GLP-1) is of particular interest because of its ability to regulate blood glucose in Type 2 diabetes, decrease appetite, reduce food intake and

Abbreviations: AP, area postrema; Arc, arcuate nucleus; BBB, blood brain barrier; BIN, behaviours indicative of "nausea"; BNST, bed nucleus of the stria terminalis; CC, cingulate cortex; CeA, central nucleus of amygdala; CSF, cerebrospinal fluid; DHM, dorsal medial nucleus of hypothalamus; Ex-4, exendin-4; Ex-(9–39), exendin-(9–39); GLP-1, glucagon-like peptide-1; ICC, interstitial cells of Cajal; i.c.v., intracerebroventricular; NTS, nucleus tractus solitarius; OVLT, organum vasculosum lamina terminae; PVN, paraventricular nucleus of hypothalamus; SFO, subfornical organ; SON, supraoptic nucleus; VMH, ventral medial nucleus of hypothalamus.

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cause weight loss (Buse et al., 2013; Meier, 2012; Secher et al., 2014; van Bloemendaal et al., 2014). Research and clinical utility are enhanced by the increasing availability of non-peptide, small molecule GLP-1 receptor agonists (e.g. exendin-4). In addition, the availability of selective GLP-1 receptor antagonists, such as exendin (9–39) (Goke et al., 1993; Kanoski et al., 2011), has facilitated identification of the sites at which GLP-1 receptor agonists act. GLP-1 receptors have been implicated in emesis induced by the cytotoxic agent cisplatin in *Suncus murinus* (Chan et al., 2013; Chan et al., 2014) and cisplatin-induced kaolin consumption in the rat (De Jonghe et al., 2016) which is argued to be an indicator of nausea in species such as the rat which lack an emetic reflex (Andrews and Sanger, 2014; Horn et al., 2013).

As part of a study investigating the site(s) at which GLP-1 receptor agonists act to induce both their desirable effect of reduced food intake, and their undesirable adverse effects of nausea, vomiting and hypertension, we have investigated the effect of both peripheral and central administration of the selective GLP-1 receptor agonist, exendin-4, in *Suncus murinus* (Chan et al., 2013) and the peripheral administration exendin-4 in the ferret (Lu et al., 2014). *Suncus murinus* and ferrets are used for these studies as in contrast to rodents they possess an emetic reflex (Horn et al., 2013). In *Suncus murinus*, exendin-4 given into the ventricles of the brain induced emesis more reliably than when given peripherally and the emetic effect was blocked by exendin (9–39), given as a pre-treatment via the same route (Chan et al., 2013; Chan et al., 2014). Moreover, exendin-4-induced emesis and associated behavioural changes were associated with increases of c-Fos levels in the AP, nucleus tractus solitarius (NTS), central nucleus of the amygdala (CeA), and hypothalamus (Chan et al., 2013).

The ferret has been widely used for mechanistic studies of emesis and has demonstrated translation to humans in the area of anti-emesis (Percie du Sert and Andrews, 2014). The ferret is also suitable for investigations of digestive tract and cardiovascular effects of pharmacological agents using implanted telemetry (e.g. Lu et al., 2014; Percie du Sert et al., 2009) and additionally the ferret has a behavioural repertoire that is arguably better characterised for studying "behaviours indicative of nausea" (BIN) (see Method for details and references). Studies of GLP-1 receptors and emesis initially performed in *Suncus murinus* were therefore extended to investigate i.c.v. exendin-4 and exendin (9–39) the ferret in which GLP-1 receptor immunoreactivity is known to be widely distributed in the brain (Lu et al., 2014). This study aimed firstly to characterise the emetic and behavioural effects of i.c.v. exendin-4 to identify a dose that could be used in subsequent studies using implanted telemetry. Secondly, c-Fos expression in the brain was quantified at doses of exendin-4 inducing emesis to provide insights into the nuclei likely to be involved in emesis. Having identified a dose of exendin-4 inducing emesis we then aimed to investigate the effects of exendin-(9–39) or saline vehicle given 15 min before exendin-4 (cf. Suncus protocol, (Chan et al., 2013; Chan et al., 2014)). The results provide insights into the central sites at which GLP-1 receptors are implicated in the genesis of the side effects of GLP-1 receptor agonists but the unexpected findings from the study with exendin (9–39) raise issues about reproducibility and technical issues of i.c.v. administration that have wider implications for studies of emesis.

2. Materials and methods

2.1. Animals

Castrated adult male fitch ferrets (1.51 ± 0.08 kg; $n = 37$) were obtained from Southland Ferrets (Invercargill, New Zealand). They were housed individually in observation cages (0.5 m × 0.5 m × 0.5 m) in a temperature-controlled room at 24 ± 1 °C under artificial lighting, with lights on between 06:00 to 18:00 h, and with a relative humidity of 50 ± 5%. Food (TriPro super premium chicken meal formula dog food, American Nutrition, USA) and water were available ad libitum except when indicated. All experiments were conducted under licence from the Government of the Hong Kong SAR and the Animal Experimentation Ethics Committee, The Chinese University of Hong Kong.

2.2. Cannulation of the third ventricle

Animals were fasted overnight with free access to water. They were injected with buprenorphine (0.05 mg/kg, s.c. Temgesic®), and anaesthesia was induced by ketamine (20 mg/kg i.m.; Alfasan, Holland) to enable intubation (2/0 tube) to permit anaesthesia with 1.5% isoflurane (Halocarbon Products Corporation, USA) in oxygen (Narkomed 2C, Drager, USA). Rectal temperature was monitored and maintained at 37 °C (UCI#390 Analogue moist heating pad, Rebirth Medical & Design, Inc., Taiwan). The level of anaesthesia was monitored during surgery by the absence of the limb withdrawal reflex. Ferrets were then placed into a stereotaxic frame equipped with custom-made ear-bars and mouthpieces (David Kopf Instruments, Tujunga, USA). The temporalis muscles were exposed via a skin incision and displaced to expose the skull. A hole was drilled in the skull: coordinates for the third ventricle: 17.3 mm anterior to lambda and at the midline. Coordinates were derived from studies in our laboratory (Rudd et al., 2006) with guide-cannula placement confirmed (dye injection and histology) and literature on the topographical anatomy of the ferret brain and skull (Lawes and Andrews, 1987). A 30-gauge cannula was then inserted into the hole in the skull, 8 mm below the surface of the dura. Two screws were fixed into the skull and dental cement was applied to secure the screws and guide cannula in place. The muscle layer was closed with a 2/0 curved cutting needle and the skin was closed with a 2/0 straight cutting needle. The wound was treated with Opsite® (Smith and Nephew, UK) silicone dressing. Guide-cannula placement was confirmed by dye injection post mortem on completion of the study.

2.3. Assessment of the effect of intracerebroventricular exendin-4 and exendin (9–39) on behaviour, food intake, and c-Fos expression in the brain

Prior to experimentation, all ferrets ($n = 37$) had free access to food and water. To determine if the effect of exendin-4 on behaviour including emesis (see below for definitions) was dose-related, animals were randomised to receive saline (15 μl, i.c.v.), or exendin-4 (3–30 nmol, i.c.v. in 15 μl saline) and additionally were randomised to one of four injection times (09.00, 10.00, 14.00 or 16.00). Doses of exendin-4 given i.c.v. were initially based upon studies in *Suncus murinus* (Chan et al., 2013; Chan et al., 2014), but also included a 10 times higher dose (30 nmol) to take into account the larger brain size and ventricular volume of the ferret. Spontaneous food and water consumption during the 60 min observation period were also measured. 60 min post exendin-4 administration, the animals that had received either saline (15 μl), exendin-4 at 10 nmol or 30 nmol i.c.v. (i.e. the groups with an emetic response) were deeply anaesthetized with pentobarbitone (40 mg/kg, i.p.) (Dorminal®, Alfasan, Woerden, Holland) and perfused via the heart with ice-cold saline (120 ml) followed by 4% paraformaldehyde in phosphate-buffered saline (PBS; 200 ml). A 60-min time period was chosen primarily as c-Fos activity is reported to peak at 60–90 min following challenges (Hoffman et al., 1993) and we also considered (based upon comparable studies in *Suncus murinus* (Chan et al., 2011)) that any emetic response to i.c.v. exendin-4 would occur within minutes of injection. Brains were removed and post-fixed in 4% paraformaldehyde overnight at 4 °C and placed in an aluminium foil container filled with O.C.T. compound (Tissue-Tek, Sakura, USA). The preparations were frozen in isopentane pre-cooled liquid nitrogen for c-Fos immunohistochemistry (see below).

Following identification of a dose of i.c.v. exendin-4 (10 nmol) that induced emesis in 75% of the animals and that produced the highest level of behaviours indicative of nausea (see below and Results Section 3.1), we then investigated the effect of exendin-(9–39) on the responses in another group of animals using a dose of exendin (9–39) (100 nmol), 10× > than the dose of exendin-4 (10 nmol); the same