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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

counted and observed at ×20 magnification. The individual performing the counts was blind to the treatments received by individual animals.

2.6. Data analysis

Emetic episodes, locomotor activity data, and food and water intake were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests or a Bonferroni multiple comparison tests. Latency data to first emetic episode were assessed by a Kruskal–Wallis test followed by Dunn's multiple comparison tests (GraphPad Prism version 5.0, Inc. Version, California, USA). When an animal failed to exhibit retching and/or vomiting, a latency value equal to the test period observation time (i.e. 60 or 120 min) was used to perform the statistical analysis. c-Fos positive cell counts were analysed using one-way ANOVA followed by Bonferroni multiple comparison tests (GraphPad Prism version 5.0, Inc. Version, California, USA). ED_{50} values of exendin-4 were estimated by linear regression analysis using GraphPad Prism version 5.0 (GraphPad Software, Inc. Version, California, USA). Data are expressed as the mean ± s.e.m., unless otherwise stated. In all cases, difference between treatment groups was considered significant when $p < 0.05$.

2.7. Drug formulation

Exendin-4 and exendin (9–39) amide (American peptides, CA, USA) were dissolved in saline (0.9% w/v NaCl, 154 mM).

3. Results

3.1. General observations

No retching or vomiting was observed during the habituation period, and there were no significant differences in the locomotor activity of the animals randomised to the treatment groups prior to drug/vehicle administration ($p > 0.05$, data not shown).

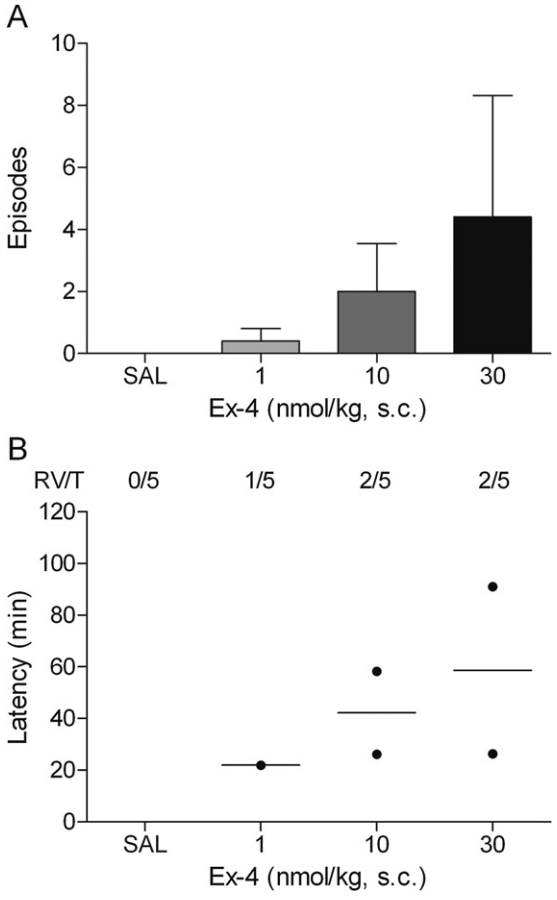

Fig. 1. Effect of subcutaneous administration of exendin-4 (Ex-4) to induce retching and/or vomiting in *S. murinus*. Results represent the mean ± s.e.m. of 5 observations during a period of 120 min. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is indicated as a fraction of each treatment group. Latency data are shown as individual responses, with lines indicating the median response time. Ex-4 (1–30 nmol/kg, s.c.) induced emesis in *S. murinus*.

3.2. Peripheral drug administration studies

The subcutaneous administration of saline was not associated with retching or vomiting. Exendin-4 induced retching and/or vomiting in a dose-dependent manner, but the latency to the first episode appeared highly variable, ranging between 21.9 and 91.0 min ($p > 0.05$, $n = 5$; Fig. 1B). Episodes of retching and/or vomiting were observed in 20% of animals at 1 nmol/kg; whereas exendin-4 induced retching and/or vomiting in 40% of animals at 10 and 30 nmol/kg, with 2.0 ± 1.5 and 4.4 ± 3.9 episodes, respectively ($p > 0.05$, $n = 5$; Fig. 1A). The ED_{50} value thus obtained was 11.5 nmol/kg, s.c. In these studies, exendin-4 inhibited significantly food ($p < 0.05$, $n = 5$) and water intake ($p < 0.001$, $n = 5$), but it failed to modulate the latency to eat and drink ($p > 0.05$, $n = 5$). Exendin-4 at 10 and 30 nmol/kg also reduced the locomotor activity (2-h total distance travelled) compared with control animals by 41.3% and 41.6% ($p > 0.05$, $n = 5$; Table 1), respectively.

3.3. Intracerebral drug administration studies

The minimum effective dose of exendin-4 to induce emesis was 0.1 nmol, which comprised of 5.3 ± 3.2 episodes of retching and/or vomiting ($p > 0.05$, $n = 6$; Fig. 2A). Exendin-4 at 3 nmol i.c.v. induced emesis in all animals within 7.6 min (25th percentile = 2.1, 75th percentile = 43.3 min; $p < 0.01$, $n = 6$; Fig. 2B), and comprised of 24.8 ± 7.3 episodes ($p < 0.01$, $n = 6$; Fig. 2A). The ED_{50} value was 5.3 nmol. Exendin-4 (0.03–3 nmol, i.c.v.) also inhibited food intake ($p < 0.001$, $n = 6$) and water intake ($p < 0.01$, $n = 6$) and reduced locomotor activity significantly by up to 85% ($p < 0.05$, $n = 6$; Table 1).

In another set of experiments, exendin-4 at 3 nmol i.c.v., induced 6.3 ± 1.9 episodes of emesis ($p < 0.05$ compared with all

Table 1
Effect of exendin-4 and exendin (9–39) on spontaneous behaviours in *S. murinus*. Data represent the mean ± s.e.m. of 5–8 experiments. Exendin-4 inhibited food and water intake significantly following both subcutaneous and intracerebroventricular administration. Exendin (9–39) had no effect on food and water intake and was ineffective in reversing the exendin-4-induced inhibition of food and water intake. Exendin-4 had no effect on velocity but reduced distance travelled by the animals significantly following intracerebroventricular administration.

Treatment	Feeding behaviour		Locomotor activity	
	Food intake (g)	Water intake (g)	Distance travelled (m)	Velocity (m/s)
Exendin-4 (10–30 nmol/kg, s.c.)				
Saline	1.6 ± 0.1	3.7 ± 0.3	23.9 ± 6.1	0.4 ± 0.05
1 nmol/kg	1.4 ± 0.1*	3.3 ± 0.3	24.2 ± 4.8	2.8 ± 0.3
10 nmol/kg	0.3 ± 0.03***	1.4 ± 0.3***	14.0 ± 3.1	1.9 ± 0.3
30 nmol/kg	0.2 ± 0.05***	0.7 ± 0.2***	13.9 ± 2.1	2.0 ± 0.5
Exendin-4 (0.3–3 nmol, i.c.v.)				
Saline	0.5 ± 0.2	1.2 ± 0.3	37.1 ± 16.8	2.4 ± 1.2
0.03 nmol	0***	0***	2.4 ± 0.8**	0.6 ± 0.09
0.1 nmol	0***	0***	3.3 ± 1.4**	0.7 ± 0.08
0.3 nmol	0***	0.1 ± 0.1**	4.2 ± 1.0*	2.4 ± 1.2
1 nmol	0***	0.03 ± 0.03**	5.3 ± 2.7*	2.7 ± 1.5
3 nmol	0***	0***	2.3 ± 0.8**	1.3 ± 0.4
Exendin (9–39) (30 nmol, i.c.v.) and exendin-4 (3 nmol, i.c.v.)				
Saline/saline	0.4 ± 0.1	0.7 ± 0.2	8.1 ± 3.0	1.8 ± 0.5
Saline/exendin-4	0	0	3.7 ± 1.3	1.3 ± 0.3
Exendin (9–39)/saline	0.3 ± 0.07	0.7 ± 0.2	13.1 ± 3.9	3.4 ± 1.7
Exendin (9–39)/exendin-4	0	0	5.3 ± 2.7	0.9 ± 0.2

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared with saline treated animals.

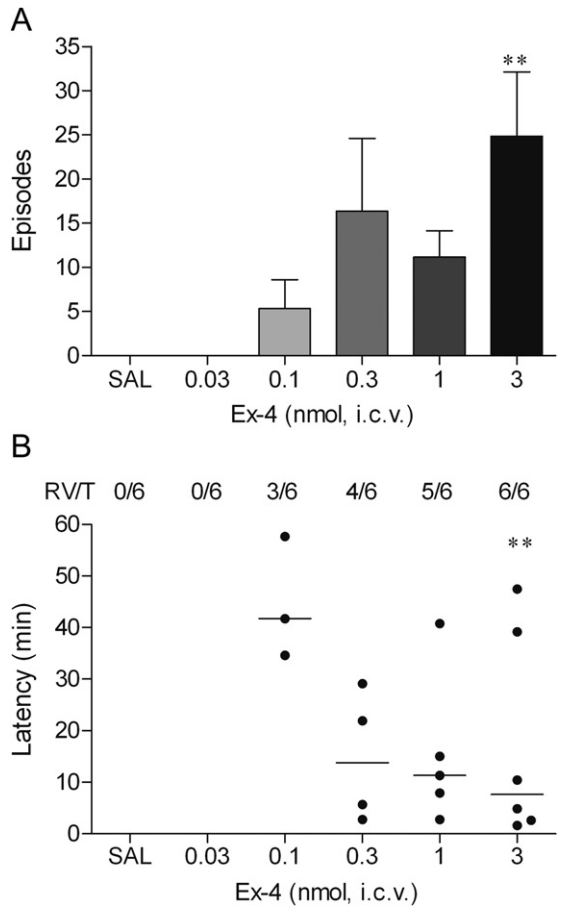

Fig. 2. Effect of intracerebroventricular administration of exendin-4 (Ex-4) to induce retching and/or vomiting in *S. murinus*. Results represent the mean ± s.e.m. of 6 determinations during a period of 60 min. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is indicated as a fraction of each treatment group. Latency data are shown as individual responses, with lines indicating the median response time. Ex-4 (0.03–3 nmol, i.c.v.) induced emesis in *S. murinus* significantly. $^{**}p < 0.01$ compared with saline treated group.

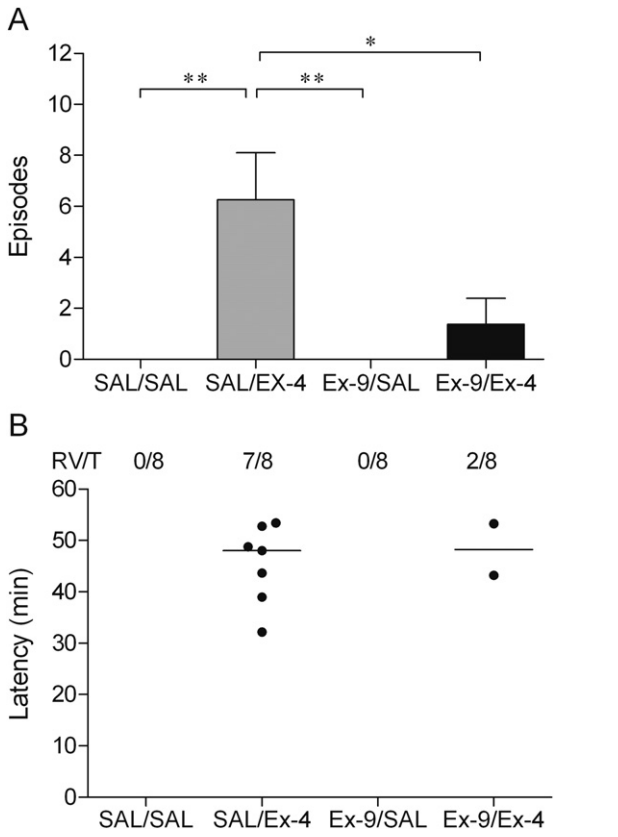

Fig. 3. The effect of intracerebroventricular administration of exendin (9–39) (Ex-9) on exendin-4 (Ex-4)-induced retching and/or vomiting in *S. murinus*. Results represent the mean ± s.e.m. of 8 determinations during a period of 60 min. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is indicated as a fraction of each treatment group. Latency data are shown as individual responses, with lines indicating the median response time. Ex-9 (30 nmol, i.c.v.) antagonised Ex-4- (3 nmol, i.c.v.) induced emesis (A) but did not affect the latency to vomiting (B). $^{*}p < 0.05$ and $^{**}p < 0.01$ between treatment groups.

other groups, $n = 8$; Fig. 3A) following a median latency of 48.4 min (25th percentile = 39.0, 75th percentile = 59.8 min; $p > 0.05$, $n = 8$; Fig. 3B), inhibited food and water intake ($p < 0.01$, $n = 8$), and reduced locomotor activity by 54.1% ($p > 0.05$, $n = 8$; Table 1). Representative photomicrographs of c-Fos expression within the hindbrain are shown in Fig. 4. On inspection of the brains, it was found that exendin-4 had increased the number of c-Fos positive cells in the AP, NTS, as well as several hypothalamic regions, including the CeA, PVH, PLH, VMH, and, DMH ($p < 0.01$, $n = 5$, Fig. 5).

Exendin (9–39) alone failed to induce emesis, affect food and water intake, or alter locomotor activity ($p > 0.05$, $n = 8$; Table 1), and it also failed to induce c-Fos expression in the brain areas examined ($p > 0.05$, $n = 5$; Figs. 4 and 5). However, pretreatment with exendin (9–39), 30 nmol, i.c.v., significantly reduced exendin-4-induced emesis by 80.4% ($p < 0.05$, $n = 8$; Fig. 3A), but failed to affect the latency to the first episode of retching and/or vomiting (median latency 48.2, 25th percentile = 43.2, 75th percentile = 53.3 min; $p > 0.05$, $n = 8$; Fig. 3B), or the inhibition of food and water intake ($p > 0.05$, $n = 8$); but reduced the locomotor activity by 35.2% ($p > 0.05$, $n = 8$; Table 1). On examination of the brains it was found that exendin (9–39) reduced the numbers of c-Fos positive cells induced by exendin-4 in the AP and NTS by 38.1% ($p < 0.05$, $n = 5$) and 53.2% ($p < 0.01$, $n = 5$), respectively. Similarly, exendin (9–39) antagonised the exendin-4-induced increase in the number of c-Fos positive cells in the CeA and PVH by 45.9% ($p > 0.05$, $n = 5$) and 48.9% ($p < 0.05$, $n = 5$; Fig. 5), respectively. There were also >50% reductions in the PLH, VMH, and DMH ($p < 0.05$, $n = 5$). However, the exendin-4-induced increase in c-Fos positive cells in the Arc was not antagonised by exendin (9–39) ($p > 0.05$, $n = 5$; see Fig. 5).

4. Discussion

The importance of the present studies was to reveal for the first time the anti-emetic action of the GLP-1 receptor antagonist, exendin (9–39). The studies also confirmed clinical findings that the GLP-1 receptor agonist, exendin-4, induces emesis following peripheral administration (Buse et al., 2011). The present study showed that exendin-4 was also emetic following i.c.v. administration, suggesting a mechanism to activate GLP-1 receptors in the brain.

Since one therapeutic uses of GLP-1 receptor agonists is to treat diabetes (see Introduction), we had previously determined the potency of exendin-4 to reduce glucose levels in *S. murinus*. Exendin-4 had significant activity in reducing blood glucose levels (following a glucose load) at 10 nmol/kg, s.c. and 1 nmol, i.c.v. The effect of centrally administered exendin-4 to reduce blood glucose was prevented by exendin (9–39) at 10 nmol, i.c.v. (Chan et al., 2011). In the present studies, the emetic action of exendin-4 was

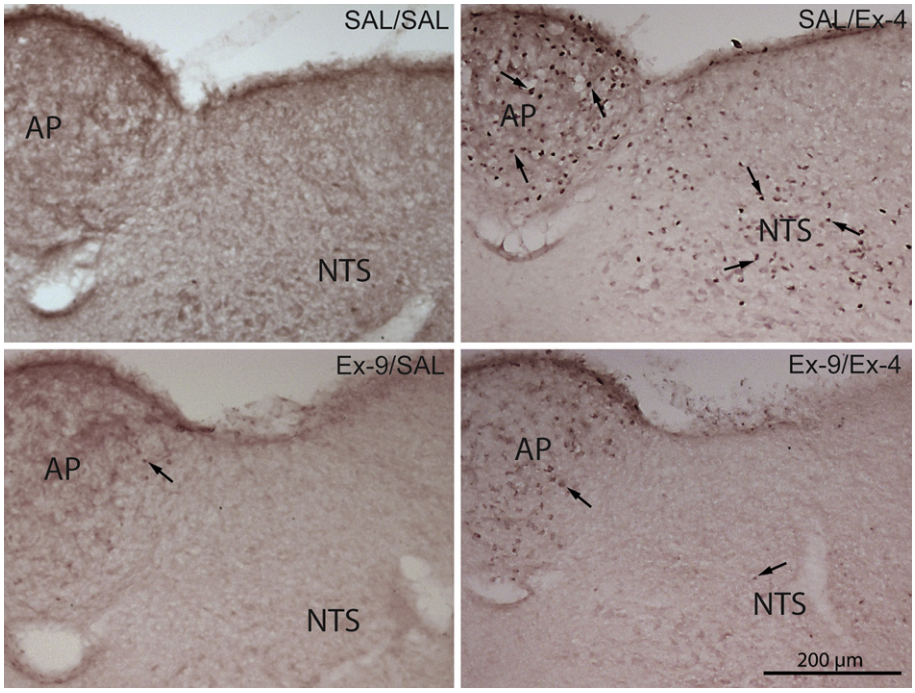

Fig. 4. Representative photomicrographs illustrating c-Fos expression (violet nuclear label) in the caudal brainstem after i.c.v. administration of exendin (9–39) and exendin-4. Arrows point out some of the many activated c-Fos positive cells. AP = area postrema; NTS = nucleus tractus solitarius. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

seen at 10–30 nmol/kg, s.c. in 40% of animals, following a latency of approximately 30 min, which is consistent with the delay in reducing blood glucose levels (Chan et al., 2011). Comparatively, the lowest dose required to induce emesis following i.c.v. administration was 0.1 nmol, with the onset of emesis being more rapid and consistent (~5 min) when the dose was increased to 3 nmol. Most importantly, exendin (9–39) (10 nmol, i.c.v.) prevented the emesis induced by exendin-4, implicating GLP-1 receptors in the mechanism of action.

The versatility of c-Fos expression as a marker for the activation of neuroendocrine neurons by various nauseagenic treatments has been well documented. Consistent with studies in rats (Van Dijk et al., 1997), exendin-4 induced c-Fos expression in the NTS and AP of *S. murinus*, and this effect was antagonised by exendin (9–39) at 30 nmol, i.c.v. Indeed, the pattern of c-Fos expression was similar to that induced by lithium and cholecystokinin in the rats and ferrets (Billig et al., 2001; Swank et al., 1995). These findings suggest that these nauseagenic treatments and exendin-4 may activate similar central neural circuits.

It was clear from our studies that exendin-4 could induce c-Fos expressions in specific hypothalamic nuclei, including the CeA, PVH, VMH, DMH, and PLH, with the effects antagonised by exendin (9–39). GLP-1 receptor immunoreactivity has been detected in all these brain regions (Chan et al., 2011; Goke et al., 1995; Merchenthaler et al., 1999). Interestingly, however, injection of exendin-4 into the medial NTS, but not the amygdala, induces kaolin consumption in rodents (Kanoski et al., 2012) to more heavily implicate the brainstem nuclei in the ingestive response to exendin-4. However, it is possible that pica and emesis may involve different brain circuitry. For example, *S. murinus* vomits, but it does not consume kaolin following several emetic/nauseagenic challenges (Yamamoto et al., 2004).

The effect of peripherally administered exendin-4 to induce pica in rats is independent of the abdominal vagal system (Kanoski et al., 2012). Further, Tang-Christensen et al. (1996) have reported that central, but not peripheral, administration of GLP-1 significantly inhibits feeding behaviour, whereas both central and peripheral administration of GLP-1 inhibit drinking behaviour. In humans, s.c. administration of exendin-4 reduces calorie intake and increases feeling of satiety (Meier et al., 2002). In the present studies, exendin-4 (1–30 nmol/kg s.c.) profoundly reduced food and water intake at 10 and 30 nmol/kg. Following i.c.v. (0.03–3 nmol) administration, food and water intake were completely inhibited at all doses. It is interesting that relative to regions of the hypothalamus, exendin-4 failed to produce a statistically significant increase in c-Fos expression in the Arc, although this brain area is thought to play a pivotal role in the integration of signals that regulate appetite (Wynne et al., 2005).

It is of major interest that the effects of exendin-4 on food and water consumption were not reversed by exendin (9–39), whereas many of the effects on c-Fos expression in the brainstem and hypothalamic regions were. It may be pertinent that GLP is known to have 'non-classical' actions in terms of receptor binding, signal transduction or effects resistant to exendin (9–39). For instance, central and peripheral infusions of exendin-4 suppress changes in plasma ghrelin, an orexigenic peptide predominately secreted by the stomach, in fasted rats that are not mimicked by GLP-1, or antagonised by exendin (9–39) (Perez-Tilve et al., 2007). These findings implicate a role for a "non-classical" GLP-1 receptor coupled to the regulation of ghrelin secretion, and exendin-4 probably acts at a GLP-1 receptor distinct from the "classical" type (Perez-Tilve et al., 2007).

Finally, our experiments revealed that i.c.v. rather than s.c. administration of exendin-4 reduced locomotor activity significantly. Such a reduction in locomotor activity has been observed following central administration of GLP-1 in rats (Turton et al., 1996). It is likely that the inhibition of locomotor activity is part of the satiety sequence following nutrient ingestion (Antin et al., 1975), which may be expected to occur when an animal is also preparing to vomit.

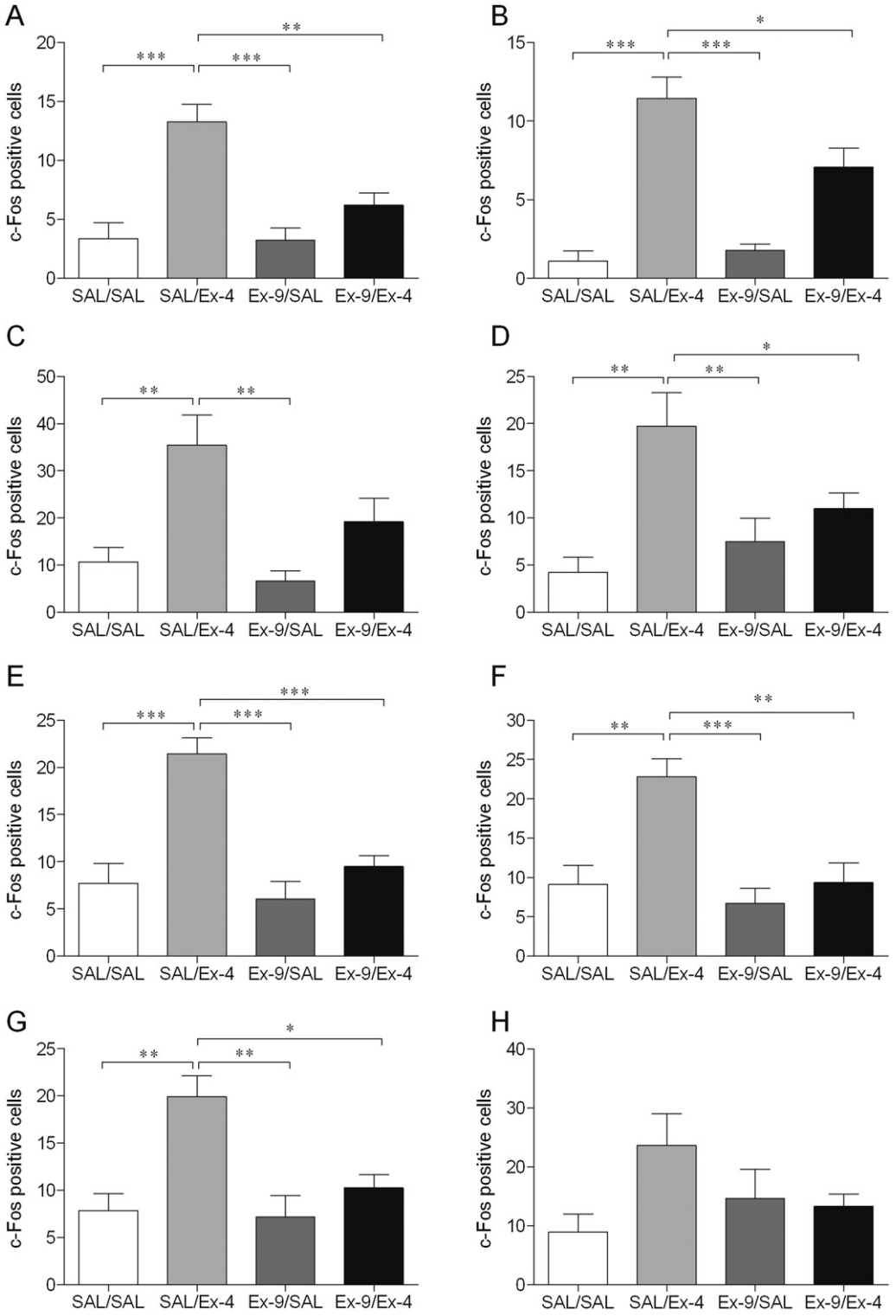

Fig. 5. Effect of exendin (9–39) (Ex-9) on exendin-4 (Ex-4) induced c-Fos expression in the CNS in *S. murinus*. Data represent the mean ± s.e.m. of 5 experiments. Ex-9 (30 nmol, i.c.v.) antagonised the increased c-Fos expressions induced by Ex-4 (3 nmol, i.c.v.) in the area postrema (A), nucleus tractus solitarius (B), central nucleus of the amygdala (C), paraventricular hypothalamus (D), peripheral lateral hypothalamus (E), ventromedial hypothalamus (F) and dorsomedial hypothalamus (G) but not arcuate nucleus (H). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared between treatment groups.

5. Conclusion

In conclusion, exendin-4 produced a number of physiological changes in *S. murinus*, including inhibition of food and water intake, induction of emesis, and reduction of locomotor activity. GLP-1 receptors in both the brainstem and hypothalamus may be critical for the side effect of emesis and loss of appetite produced by GLP-1 receptor agonists in the clinic. It is important to note that nausea and vomiting may result in loss of appetite, and it is difficult to rule out whether exendin-4 exerts its emetic effect solely via the

brainstem vomiting centre or via comprehensive pathways involving both the hypothalamus and brainstem. Effects on feeding, however, may be mediated by "non-classical" GLP-1 receptors and this requires further investigation. The anti-emetic spectrum of action of exendin (9–39) needs to be explored in order to determine whether GLP-1 receptor antagonists could have a clinical application in preventing emesis induced by other treatments.

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