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Centrally located GLP-1 receptors modulate gastric slow waves and cardiovascular function in ferrets consistent with the induction of nausea

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ABSTRACT

Glucagon-like peptide-1 (GLP-1) receptor agonists are indicated for the treatment of Type 2 diabetes and obesity, but can cause nausea and emesis in some patients. GLP-1 receptors are distributed widely in the brain, where they contribute to mechanisms of emesis, reduced appetite and aversion, but it is not known if these centrally located receptors also contribute to a modulation of gastric slow wave activity, which is linked causally to nausea. Our aim was to investigate the potential of the GLP-1 receptor agonist, exendin-4, administered into the 3rd ventricle to modulate emesis, feeding and gastric slow wave activity. Thermoregulation and cardiovascular parameters were also monitored, as they are disturbed during nausea.

Ferrets were used as common laboratory rodents do not have an emetic reflex. A guide cannula was implanted into the 3rd ventricle for delivering a previously established dose of exendin-4 (10 nmol), which had been shown to induce emesis and behaviours indicative of 'nausea'. Radiotelemetry recorded gastric myoelectric activity (GMA; slow waves), blood pressure and heart rate variability (HRV), and core temperature; food intake and behaviour were also assessed.

Exendin-4 (10 nmol, i.c.v.) decreased the dominant frequency of GMA, with an associated increase in the percentage of bradygastric power (lasting ~4 h). Food intake was inhibited in all animals, with 63% exhibiting emesis. Exendin-4 also increased blood pressure (lasting ~24 h) and heart rate (lasting ~7 h), decreased HRV (lasting ~24 h), and caused transient hyperthermia. None of the above parameters were emesis-dependent.

The present study shows for the first time that gastric slow waves may be modulated by GLP-1 receptors in the brain through mechanisms that appear independent from emesis. Taken together with a reduction in HRV, the findings are consistent with changes associated with the occurrence of nausea in humans.

1. Introduction

Glucagon-like peptide-1 (GLP-1) is secreted by the small intestine in response to nutrient ingestion (Kreymann et al., 1987), and it reduces blood glucose levels by stimulating glucose-induced insulin secretion and suppresses glucagon production (Egan et al., 2003; van Dijk and Thiele, 1999; Vella et al., 2001). Whilst synthetic GLP-1 receptor agonists are used in the treatment of Type 2 diabetes to control blood glucose, they can also reduce appetite, food intake, and cause weight loss (Buse et al., 2013; Secher et al., 2014; van Bloemendaal et al., 2014) so are also utilised for the treatment of obesity with or without diabetic co-morbidity (Lean et al., 2014; Vilsboll et al., 2012). Unfortunately, the side effect profile of GLP-1 receptor agonists in both healthy volunteers (Agerso et al., 2002) and patients includes nausea and, less frequently, vomiting (Buse et al., 2004; Heine et al., 2005; Kanoski et al., 2012; Kendall et al., 2005), suggesting that the side-effect profile is not related to the underlying disease. The mechanism is not clearly defined, although it may be related to a disturbance of gastric function. A clarification of the role of centrally located GLP-1 receptors in emetic mechanisms is therefore required to aid the rational design of novel agonists with the aim of reducing side effects.

Studies in mice, which are incapable of emesis (Horn et al., 2013; Sanger et al., 2011), have shown that anorectic doses of GLP-1 can inhibit feeding through a capsaicin-sensitive mechanism (Talsania et al., 2005), implicating abdominal vagal afferents. Yet, neurons in the area postrema (AP), nucleus tractus solitarius (NTS), amygdala, and parabrachial nucleus (Parker et al., 2013) are also implicated. Whilst doses of synthetic GLP-1 receptor agonists causing either conditioned taste aversions or pica (kaolin consumption) – both of which have been argued to be indices of nausea and/or emesis in rats (Andrews and

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Sanger, 2014; Stern et al., 2011) – also involve the same brainstem areas (AP, NTS), the mechanism appears independent of the abdominal vagus (Kanoski et al., 2012). In *Suncus murinus*, the GLP-1 receptor agonist, exendin-4, inhibits food and water consumption and induces emesis that is associated with an increases in c-Fos levels in the AP, NTS, central nucleus of the amygdala (CeA), and hypothalamus (Chan et al., 2013). Our recent studies using ferrets, which have proven translational value to humans (Percie du Sert and Andrews, 2014), have also demonstrated that the brainstem is critically involved in the mechanism of exendin-4-induced emesis, but 'behaviours indicative of nausea' probably involve the amygdala (Lu et al., 2016).

We have previously used the ferret to demonstrated the hypoglycaemic and anorectic effects of peripherally-administered exendin-4 (100 nmol/kg, s.c.) (Lu et al., 2014). The studies revealed the incidence of emesis was comparable to that observed in humans (11% vs 12.8%), with associated changes in gastric myoelectric activity (GMA; bradygastria and a reduction in power) and a reduction in heart rate variability (HRV) (Lu et al., 2014)). This is of interest as, whilst it is not possible to know if an animal is experiencing nausea, the changes in the GMA and HRV readouts are known to occur during nausea in man (Farmer et al., 2014; Kim et al., 2011; Stern et al., 2011). Since it is not known if centrally located GLP-1 receptors are involved in all of the side effects caused by GLP-1 receptor agonist therapy, the present study in the ferret was designed to investigate whether intracerebroventricularly (i.c.v.) administered exendin-4 also modulates GMA and heart rate variability, a potential biomarkers of 'nausea', in addition to its well-known action to reduce food intake and cause emesis.

A 10 nmol dose of exendin-4 was selected for this study utilising telemetry of GMA, HRV, blood pressure and core temperature in unrestrained ferrets. The exendin-4 dose was based on previous experimentation showing that it induced emesis, inhibited food intake, significantly increased 'behaviours indicative of nausea' and activated brain circuitry implicated in emetic and homeostatic control (Lu et al., 2016). We hypothesised that if this dose of exendin-4 were inducing "nausea" (as indicated by behaviour in our previous study), then it would produce changes in the GMA and HRV characteristic of those seen in humans. As exendin-4 inhibited food intake we also investigated the effects of a brief period of food deprivation (and feeding) on GMA and HRV to investigate the extent to which any effects of exendin-4 could be secondary to reduced food intake.

2. Methods

2.1. Animals

Adult male castrated fitch ferrets (1.54 ± 0.17 kg) were obtained from Southland Ferrets (Invercargill, New Zealand) and 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. The relative humidity was maintained at 50 ± 5%. Water and food (TriPro super premium chicken meal formula dog food, American Nutrition, USA) were given *ad libitum* unless otherwise stated. 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

Ferrets (n = 8) were fasted overnight, but allowed free access to water. They were then injected with buprenorphine (0.05 mg/kg, s.c. Temgesic®), and anaesthesia was induced by ketamine (20 mg/kg i.m.; Alfasan, Holland). They were intubated using 2/0 tube, and anaesthesia was maintained with 1.5% isoflurane (Halocarbon Products Corporation, USA) in oxygen using an anaesthetic machine (Narkomed 2C, Drager, USA). Rectal temperature was monitored and maintained at 37 °C using a heating pad (UCI#390 Analogue moist heating pad, Rebirth Medical & Design, Inc., Taiwan), and the level of anaesthesia was assessed and monitored throughout the surgery by the absence of the pedal withdrawal reflex. Ferrets were then placed into a stereotaxic frame equipped with custom-made ear-bars and mouth-pieces (David Kopf Instruments, Tujunga, USA). The temporalis muscles were exposed via a skin incision and displaced exposing the skull. A hole was drilled in the skull: coordinates for the lateral ventricle are 17.3 mm anterior to lambda and 0 mm lateral to the midline. Coordinates were derived from our own previous studies in the ferret (Rudd et al., 2006) and from histology and publications describing the topographical anatomy of the ferret brain and skull stereotaxic landmarks (Lawes and Andrews, 1987). A 30-gauge cannula was then inserted into the hole 8 mm below the surface of the dura. Two screws were fixed into the skull and dental cement was applied to the screws and the guide cannula. After fixation, the muscle layer was closed with a 2/0 curved cutting needle, while the skin layer was closed with a 2/0 straight cutting needle. The wound was then sprayed with silicone dressing (Opsite®, Smith and Nephew, UK). Some of the ferrets immediately received further surgery (see below). Guide-cannula placement was confirmed by dye injection post mortem upon completion of the study.

2.3. Implantation of radiotelemetry transmitters

Immediately after cannulation of the third ventricle, radiotelemetric transmitters were implanted in eight animals. Following a midline abdominal incision, the catheter of a C50-PXT transmitter (Data Sciences, Inc., USA) was inserted into the abdominal aorta up to a length of approximately 2 cm. A 2 × 2 mm piece of sterile gauze was placed over the catheter's entry point and fixed with a drop of tissue glue (3 M Vetbond Tissue Adhesive, Minnesota, USA). The body of the transmitter was sutured to the left side of the abdominal wall muscle with its biopotential wires and catheter facing caudally. The gastric antrum was exposed and the biopotential wires were inserted into the muscle and secured in place by applying serosal sutures. The abdominal cavity was sutured closed in layers and the surface wound sprayed with silicone dressing. After all surgical procedures, the ferrets were given marbofloxacin (Marbocyl®, 2 mg/kg, s.c.) once daily for 3 days, and buprenorphine (0.05 mg/kg, s.c.) dosing was repeated 8–12 h after the first dose. Animals were allowed to recover for at least 7 days prior to further experimentation. Recovery was uneventful, with animals eating and drinking normally the day after surgery. The handling stress during experiments was minimised by habituating the animals to handling by the insertion of a dummy needle into the guide cannula for 7 consecutive days prior to experimentation.

2.4. Measurement of the effect of exendin-4 (10 nmol, i.c.v.) on GMA, food intake, and on cardiovascular homeostasis and core temperature

The eight animals implanted with radiotelemetry transmitters were fasted overnight. At 08.00 h, they were presented with 20 g of food. At 08.30 h, the amount of food eaten over 30 min was measured and is expressed as g of food eaten/kg body weight (see Results). Any uneaten food was removed and the animals were fasted again. At 10.00 h (t = 0), they were randomised and injected with either sterile saline (15 µl, i.c.v.) or exendin-4 (10 nmol, i.c.v. in 15 µl saline). A crossover design was employed, with a seven-day interval. The order of treatments was randomised using a Latin square in each arm of the experiment, with animals' behaviour being recorded for 4 h. At 14.00 h (t = 4 h), ferrets were given 20 g of food and the amount eaten over the next hour (14.00 to 15.00) was measured and is expressed in the Results as g of food/kg body weight. At 15.00 h, ferrets had free access to food until the end of the experiment (10.00 h the next day). The radiotelemetric recording for cardiovascular parameters, core body temperature (CBT), and GMA were recorded

throughout the experiment. All ferrets were killed by an overdose of pentobarbitone (80 mg/kg, i.p.) at the end of the study, and the ventricular cannula placement was then subsequently confirmed.

2.5. Data analysis

(i) *Behaviour definitions*: Emesis was characterised as rhythmic abdominal contractions that were either associated with oral expulsion of solid or liquid material from the gastrointestinal tract (i.e. vomiting), or not associated with passage of material (i.e. retching). An episode of retching and/or vomiting was considered separate when the animal changed its location in the observation cage, or when the interval between retches and/or vomits exceeded 5 s (Rudd et al., 1994).

(ii) *Radiotelemetry*: Telemetric data were analysed using Spike2 (Version 7, Cambridge Electronic Design). The method for analysing telemetric GMA has been described in previous studies. Dominant power (DP) is defined as the highest power in the 0 to 15 counts per min (cpm) range, and dominant frequency (DF) is defined as the frequency bin with the highest power in the 0 to 15 cpm range (Percie du Sert et al., 2009). GMA data were also repartitioned into the percentage of time in each recording epoch spent in bradygastria, normogastria, or tachygastria (Percie du Sert et al., 2009 for definitions). Systolic blood pressure (BP) was calculated from the peak of the BP recording trace, and diastolic BP was calculated from the trough. Mean arterial BP was defined as systolic BP/3 + 2*diastolic BP/3 (Zheng et al., 2008). For heart rate (HR), the peak-to-peak interval was first calculated, and HR = 60/P-P interval (bpm). The time-domain HR variability (HRV) was calculated by taking the standard deviation of P-P intervals (SDNN) in 5 min segments (Reardon and Malik, 1996). The frequency-domain analysis was performed using the fast Fourier transform. The total power of all R-R intervals in 5-min segments was determined, along with its low frequency (LF, 0.04–0.15 Hz) and high frequency (HF, 0.15–0.7 Hz) components and LF/HF ratio (Bogucki et al., 2015). CBT was calculated by taking the average of the data at 5 min intervals. All the data were eventually averaged in 1 h segments for statistical analysis.

All statistical analyses were performed using GraphPad Prism version 5 (GraphPad Prism version 5.0, Inc., California, USA). Differences between behaviour and food consumption were compared using paired Student's *t*-test where appropriate. The differences of mean arterial BP, HR, SDNN, LF, HF, LF/HF, CBT and GMA between saline and exendin-4 treatment groups were assessed using repeated two-way ANOVA (factors: time and treatment) and Bonferroni tests. All data are expressed as mean ± s.e.m. In all cases, differences were considered significant when $P < 0.05$.

2.6. Drug formulation

Exendin-4 (American Peptide Company, Sunnyvale, CA) was dissolved in sterile saline (0.9% w/v).

3. Results

3.1. Baseline gastric, cardiovascular and temperature parameters and the effect of feeding

Examination of pooled data ($n = 8$; see Fig. 1) revealed a baseline arterial BP of 107.2 ± 0.3 mm Hg (systolic BP: 138.2 ± 0.5 mm Hg; diastolic BP: 91.6 ± 0.3 mm Hg; pulse BP: 46.6 ± 0.2 mm Hg). The basal HR was 224.0 ± 1.1 bpm, and HRV (Fig.2A) as revealed by SDNN was 0.061 ± 0.001 (arbitrary units) (Fig. 2B). The frequency-domains of HRV were LF, 760.7 ± 26.03 ms^2; HF, 3179.0 ± 128.8 ms^2; and LF/HF, 0.262 ± 0.008 (Fig. 2C-2E). Baseline CBT was 38.4 ± 0.1 °C (Fig. 3). Baseline GMA recordings revealed a DF of 9.97 ± 0.04 cpm and a DP of 0.0024 ± 0.00013 mV^2, with 14.4 ± 0.3% of power in the bradygastric range, 68.4 ± 0.9% of power in the normogastric range, and 10.5 ± 0.8% of power in the tachygastric range ($n = 8$, Fig. 4).

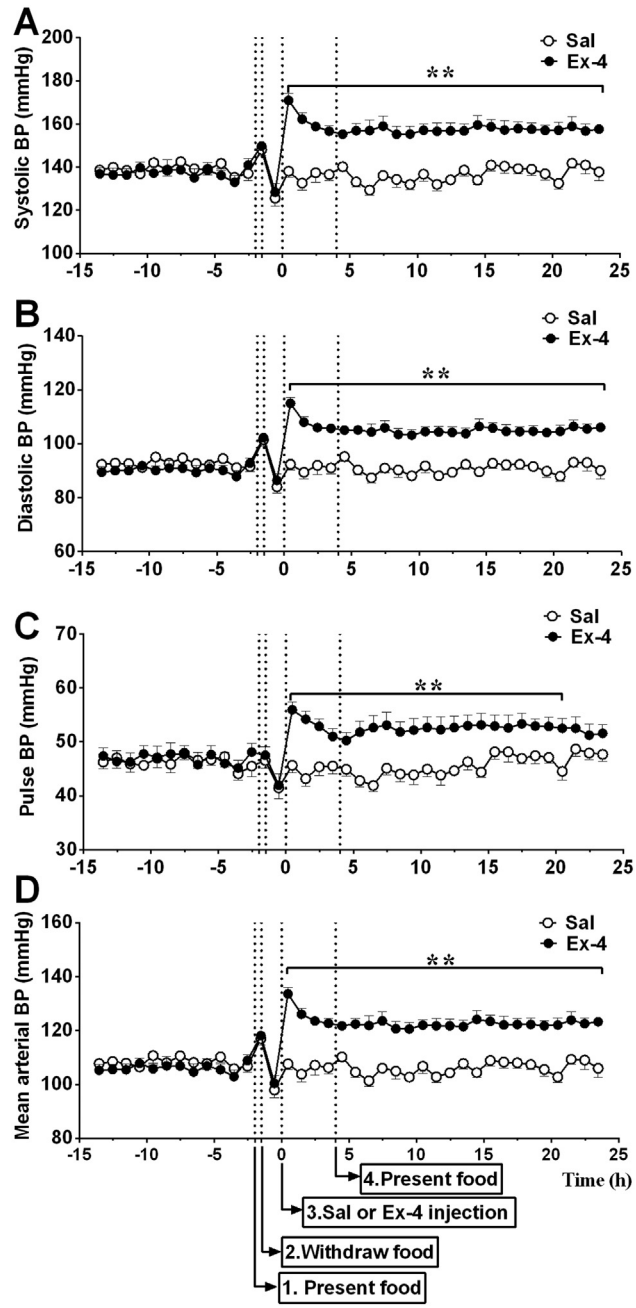

Fig. 1. Effect of i.c.v. administration of either saline (15 µl) or exendin-4 (10 nmol, i.c.v) on cardiovascular function: (A) systolic BP, (B) diastolic BP, (C) Pulse BP, and (D) mean arterial BP are shown.Note the sustained effect of exendin-4 on blood pressure. BP = blood pressure, Sal = saline, Ex-4 = exendin-4. Data represents the mean ± s.e.m. of 8 animals. Significant differences are shown as $^{**}P < 0.01$ (repeated measures two-way ANOVA followed by Bonferroni tests).

Following food consumption (animals consumed 5.3 ± 0.5 g/kg of food; see Figs. 2 and 3), there was a small transient (60 min duration) increase in BP (systolic BP: 8% increase; diastolic BP: 11% increase; pulse BP: no change; mean arterial BP: 9% increase), and an increase (15%) in HR and a reduction in HRV (SDNN, 32.0%; LF, 21.6%; HF, 23.6%). There was also a fall in DF (9%) and an increase in DP (252%), with a 40% fall in the % power of normogastria and a 30% increase in the % power of bradygastria without effects on the % power of tachygastria (Fig. 4).

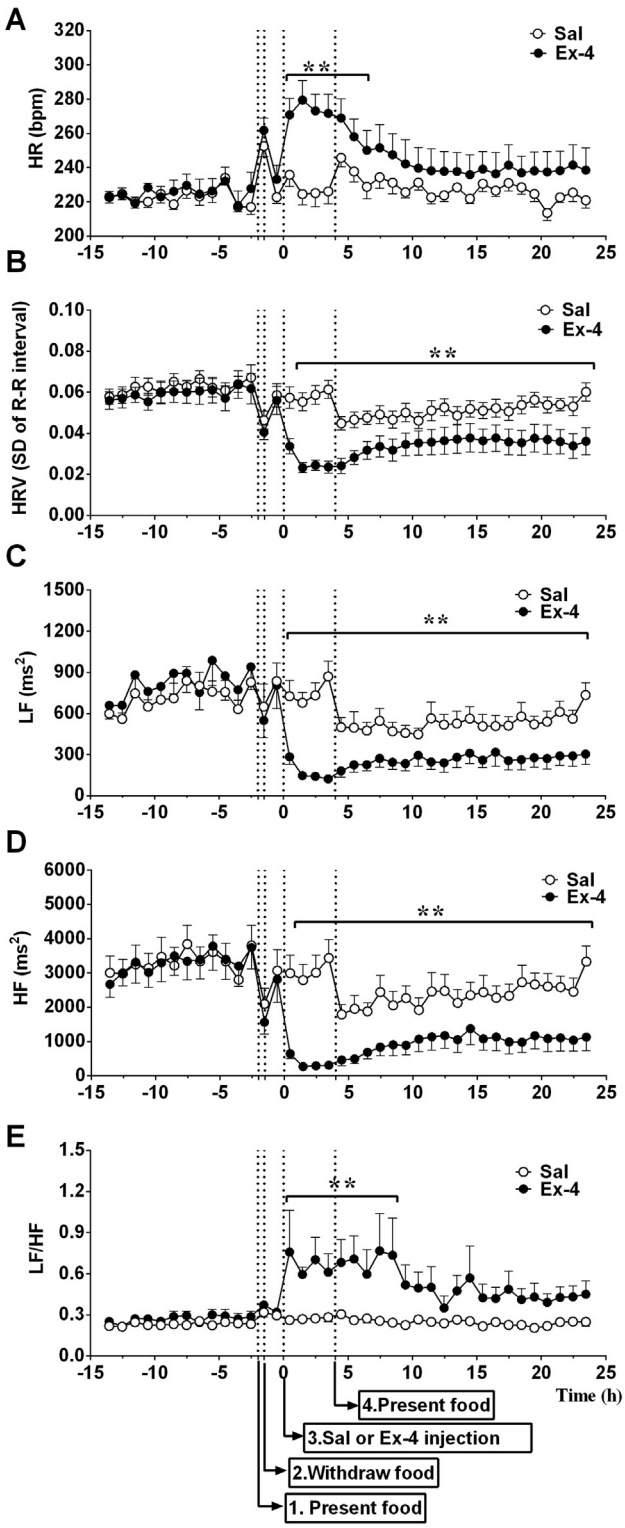

Fig. 2. Effect of i.c.v. administration of either saline (15 µl) or exendin-4 (10 nmol, i.c.v) on heart rate and heart rate variability: (A) heart rate, (B) heart rate variability (SDNN), and (C) LF HRV, (D) HF HRV, and (E) LF/HF ratio are shown. HR = heart rate (bpm), HRV = heart rate variability (SD of R-R interval), LF = low frequency, HF = high frequency, Sal = saline, Ex-4 = exendin-4. Data represents the mean ± s.e.m. of 8 animals. Significant differences are shown as $^{**}P < 0.01$ (repeated measures two-way ANOVA followed by Bonferroni tests).

3.2. *Effects of saline and exendin-4 (10 nmol, i.c.v) on emesis, food intake, GMA, CBT and the cardiovascular system*

i) *Emesis, cardiovascular parameters, and core temperature*. The administration of saline (15 µl, i.c.v.) did not induce emesis or affect the cardiovascular parameters measured, and it also failed to affect CBT or GMA ($P > 0.05$; $n = 8$, Figs. 1–4). However, administration of exendin-4 (10 nmol, i.c.v.) induced emesis in 5 out of 8 ferrets with a median latency of 36.6 min (range: 32.4–187.8 min). In the animals that responded, there were 9.1 ± 3.5 episodes of 44.0 ± 16.8 retches and 7.6 ± 3.0 vomits ($P < 0.05$). Exendin-4 also increased significantly mean arterial BP by 17.4% (systolic BP: 15.9% increase; diastolic BP: 17.9% increase; pulse pressure: 13.5% increase) and decreased HRV (SDNN) by 57.4% ($P < 0.05$). These effects were apparent within 1 h of administration and lasted up to 24 h ($P < 0.01$; $n = 8$, Fig. 1 and Fig. 2B). There was also a 20.7% increase in HR, which also was apparent within the first hour of administration and this effect lasted up to 7 h ($P < 0.01$; $n = 8$, Fig. 2A). Further frequency-domain analysis of HRV revealed that exendin-4 administration markedly decreased both LF (−82.5%) and HF (−89.6%) components of HRV, and this effect lasted up to 24 h ($P < 0.01$; $n = 8$, Fig. 2C and D). In addition, there was also a transient increase in LF/HF ratio, which lasted up to 7 h ($P < 0.01$, $n = 8$, Fig. 2E). Conversely, exendin-4 transiently increased (0.43 °C) CBT during the first 4 h after exendin-4 administration ($P < 0.05$, $n = 8$, Fig. 3). A more detailed analysis between the vomiting ($n = 5$) and non-vomiting animals ($n = 3$) did not reveal differences between BP/HR parameters (HR, HRV: SDNN, LF, HF and the LF/HF ratio) or CBT (data not shown).

ii) *GMA*. Saline treatment did not affect any of the GMA parameters, but exendin-4 (10 nmol, i.c.v) reduced DF and DP (saline: DF 9.77 ± 0.18 cpm vs exendin-4: DF 9.44 ± 0.17 cpm; saline: DP 0.0044 ± 0.0012 mV^2 vs exendin-4: DP 0.0022 ± 0.0007 mV^2; $P < 0.05$; $n = 8$) for 4 h, and there was a 45% increase in the % power of bradygastria, and a 33% reduction in the % power of normogastria ($P < 0.05$; $n = 8$, Fig. 4). The % power of the tachygastric range was unaffected compared with the saline group ($P > 0.05$; $n = 8$, Fig. 4).

Analysis of data around and during exendin-4-induced emetic episodes revealed a 43% decrease in the % power of normogastria, with an associated increase in both the % power of bradygastria (31%) and tachygastria (150%) during emesis ($P < 0.05$; $n = 5$, Fig. 5). However, there were no overall significant differences in DF, DP or the % power of bradygastria, normogastria, or tachygastria, in the animals that vomited in response to exendin-4 (10 nmol, i.c.v.) when compared with those that did not (data not shown).

iii) *Food intake*. Before exendin-4 (10 nmol, i.c.v.) or saline treatment, there was no difference in 0.5 h baseline food consumption between saline- and exendin-4- treated animals (saline: 5.2 ± 0.5 g/kg vs exendin-4: 5.4 ± 0.6 g/kg). At 4 h after i.c.v. exendin-4 or saline treatment, the saline-treated animals consumed 5.7 ± 0.6 g/kg of food, and there was a consequent decrease (~9%) in DF from 9.86 ± 0.02 cpm to 8.55 ± 0.04 cpm and a 40% reduction in the % power of normogastria ($P < 0.05$; Fig. 4). There was also an increase in the % power of bradygastria from 24 to 46% ($P < 0.01$; $n = 8$, Fig. 4), but no change in the % power of the tachygastric range. The exendin-4-treated animals did not eat and consequently there were no further changes in any of the parameters of GMA.

4. Discussion

The present study revealed for the first time that central GLP-1 receptor activation produces a change in the pattern of the gastric myoelectric activity in the ferret that is similar to that recorded in humans reporting nausea (Stern et al., 2011). Centrally administered exendin-4 also produced a long lasting elevation of BP and HR. Although the latter was not unexpected as hypertension is induced

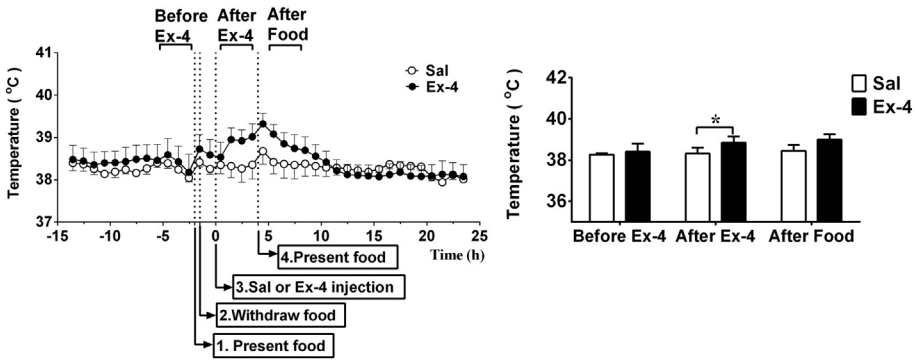

Fig. 3. Effect of i.c.v. administration of either saline (15 µl) or exendin-4 (10 nmol, i.c.v) on core body temperature. Sal = saline, Ex-4 = exendin-4. Data represents the mean ± s.e.m. of 8 animals. Significant differences are shown as $^*P < 0.05$ (paired *t*-test).

following peripheral exendin-4 administration (Lu et al., 2014), the finding of hypertension in the ferret (a carnivore) further suggests that there is a species-specific modulation of BP by GLP-1 receptor agonists (pressor responses are reported in rats (a rodent) but not in the dog (carnivore), calves (ungulate) or humans (primate); see review by Goodwill et al. (2014)). The present discussion will focus on the potential mechanisms underlying the GMA, food intake, and cardiovascular changes before reviewing the significance of the present results in understanding nausea and vomiting as adverse effects of GLP-1 receptor agonists. The limitations of the study are discussed in Section 4.5 below.

4.1. GMA

In general, exendin-4 reduced the DF and DP of the gastric slow waves and caused a shift in power from the normogastric to the bradygastric range, with little effect on the tachygastric range. However, the peri-emesis pattern showed a *decrease* in the % power of normogastria and associated increases in both the % power of bradygastria and tachygastria. These changes are similar to our previous studies where exendin-4 was administered subcutaneously (Lu et al., 2014), and yet different from those seen following feeding (Lu et al., 2016); present study) when animals are presumably not experiencing gastric distress or malaise.

The activation of gastric vagal efferents driving enteric cholinergic nerves potentiates the GMA amplitude and stabilizes the interstitial cells of Cajal (ICC) slow wave frequency. Conversely, the activation of sympathetic efferents reduces the GMA amplitude (Hinder and Kelly, 1977). Injections were given in the ventricular system and there are at least three ways in which i.c.v. exendin-4 could affect GMA, and these are not mutually exclusive: (i) direct modulation of efferent autonomic pathways from the brain to the periphery (e.g. hypothalamic modulation of the vagal outflow from the dorsal motor vagal nucleus and/or pre-sympathetic neurons in the ventrolateral brain stem); (ii) sites integrating afferent inputs and their link to efferent outputs (e.g. NTS modulated by an action of exendin-4 on GLP-1 receptors in the AP); (iii) an action to modulate hormone release (e.g. vasopressin) from the posterior pituitary (Malendowicz et al., 2003). The pattern of brain c-Fos activation with the same dose of i.c.v. exendin-4 is consistent with these potential sites of action as a significant increase in activity was found in the AP, NTS, and several regions of the hypothalamus including the paraventricular nucleus (Lu et al., 2016).

For the GMA, the reduced DF and DP of the slow waves and the shift in power from the normogastric to the bradygastric range (with some tachygastria during emesis) indicates a change from vagal to sympathetic dominance on the activity of the gastric ICC. Such a pattern of autonomic activity has been reported to occur during nausea in humans (Stern et al., 2011). Although no studies have investigated the effect of vasopressin on GMA in ferrets, dysrhythmia and bradygastria have been reported in the dog following its peripheral administration (Chen et al., 2003; Song et al., 2006), the effects reported here could involve vasopressin (or other mediators) being released from the pituitary into plasma. This would also coincide with the well-known action of vasopressin in the elevation of BP via actions on the periphery or by increasing sympathetic outflow from the central nervous system (assessed by the LF/HF ratio), which is reduced by the V1b receptor antagonist, SSR149415 (Milutinovic-Smiljanic et al., 2013).

4.2. Hypertension, tachycardia and HRV

The effect of exendin-4 (10 nmol, i.c.v.) on cardiovascular function was striking in that all animals exhibited marked and protracted hypertension (up to 24 h), with an accompanying tachycardia (lasting 7 h). This was not entirely unexpected since subcutaneously administered exendin-4 caused hypertension in the ferret (Lu et al., 2014), albeit the effect was not as large or as protracted as in the present study. Exendin-4 has also been reported to increase BP and HR in rats by modulating sympathetic outflow (Gardiner et al., 2008; Yamamoto et al., 2002) and in mice HR appears to increase via a mechanisms involving a reduction in cardiac vagal tone (Griffioen et al., 2011). However, hypertensive effects of GLP-1 receptor agonists are not reported in the dog, calf, or human (Goodwill et al., 2014). Whilst it is difficult to compare studies directly because of differences in dose, route, and ligand, the present study demonstrates that a hypertensive response to a GLP-1 receptor agonist (given centrally or peripherally) is unrelated to the ability of the species to vomit (cows, dogs, and humans can all vomit (Sanger et al., 2011).

In the present studies, exendin-4 (10 nmol, i.c.v.) produced long lasting elevations of BP and HR associated with a concomitant decrease in HRV (SDNN). Tachycardia is expected if there is a removal of parasympathetic tone and/or an increase in sympathetic tone in the heart. A power spectral analysis method was used to partition total variance of beats of the heart into frequency components. The high frequency peak (HF) is believed to reflect cardiac parasympathetic activity, whilst the low frequency peak (LF) is assumed to have a more dominant sympathetic component. Reductions in both components were observed with an overall increase in the LF/HF ratio, indicating a shift to “sympathetic dominance” (Billman, 2013).

It is notable that for GMA, HR (including HRV) and BP, the changes recorded in response to central exendin-4 are all consistent with a decrease in vagal outflow to the heart and stomach with a concomitant increase in sympathetic drive. Additional studies are required to identify the site(s) at which exendin-4 is producing this effect, as an action at either the hypothalamus or the NTS could be responsible (Coote and Chauhan, 2016). In addition, the release of vasopressin from the posterior pituitary could also contribute to both the GMA and BP changes, although in the case of the latter its protracted nature raises the issue of whether vasopressin secretion would sustain for the entire

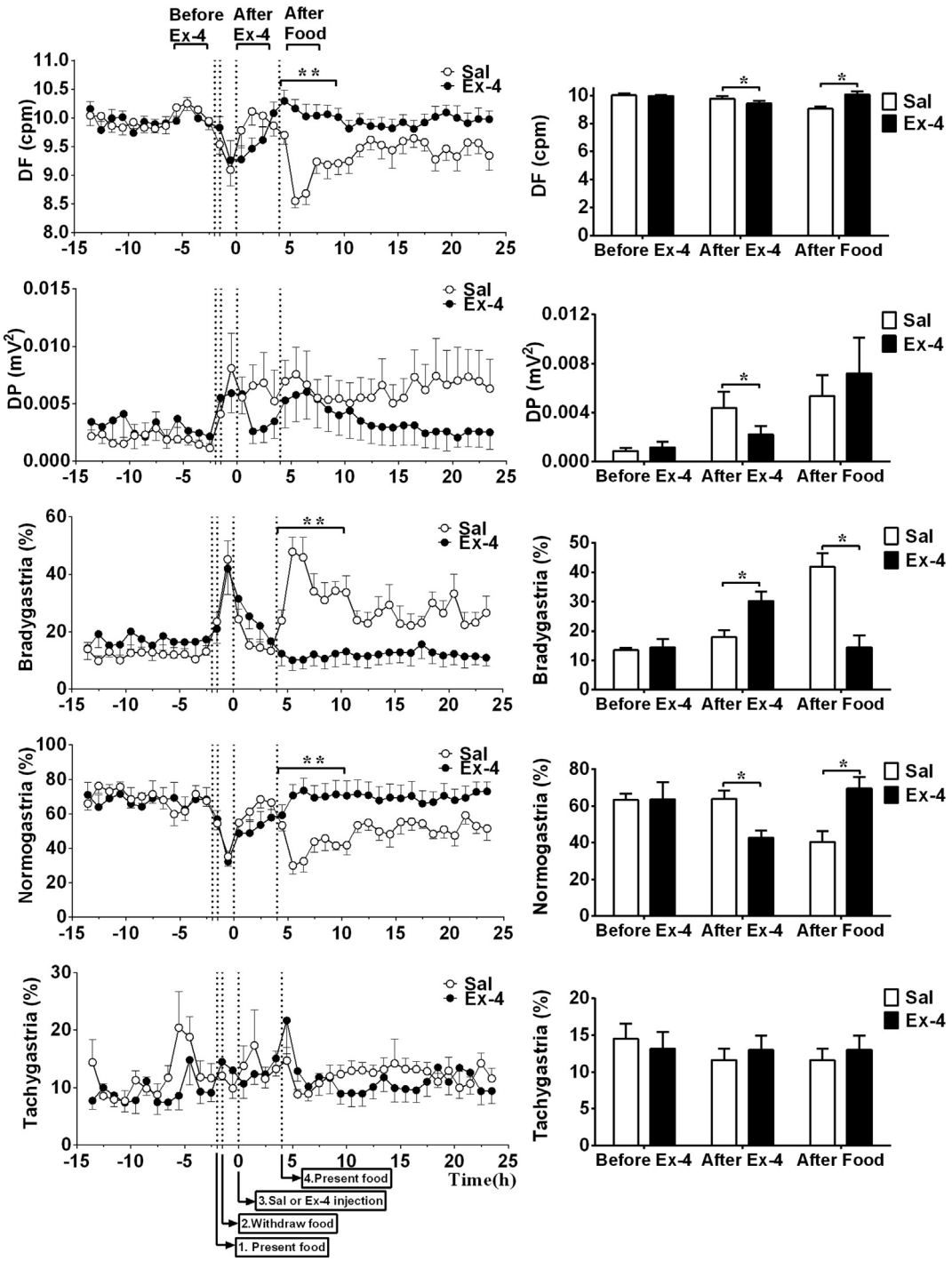

Fig. 4. Effect of i.c.v. administration of either saline (15 μl) or exendin-4 (10 nmol) and food intake on gastric myoelectric activity. DF = dominant frequency, DP = dominant power. Data represents the mean ± s.e.m. of 8 animals. Significant differences are shown as $^{**}P < 0.01$ (repeated measures two-way ANOVA followed by Bonferroni tests); $^{*}P < 0.05$ (paired *t*-test).

duration of hypertension. Serial measurements of plasma vasopressin could resolve this issue.

4.3. Hyperthermia

The effect of exendin-4 on hyperthermia is more difficult to explain, as our previous studies using ferrets did not yield any effect (Lu et al., 2014). However, in rats, peripheral and fourth ventricular administrations of exendin-4 cause hypothermia, along with an inhibition of feeding, a delay in gastric emptying, and tachycardia, all of which do not seem to involve the forebrain (Griffioen et al., 2011). Initially, the difference in thermal response to exendin-4 between the rat and the ferret was considered secondary to the physical activity of vomiting in the ferret, since a rat is incapable of vomiting. But when the data between ferrets with or without emesis were compared, no differences in temperature, as well as other measured parameters, could be found. Indeed, in man, exendin-4 administered subcutaneously is not known to have effects on body temperature at doses that cause nausea and vomiting (Buse et al., 2004).

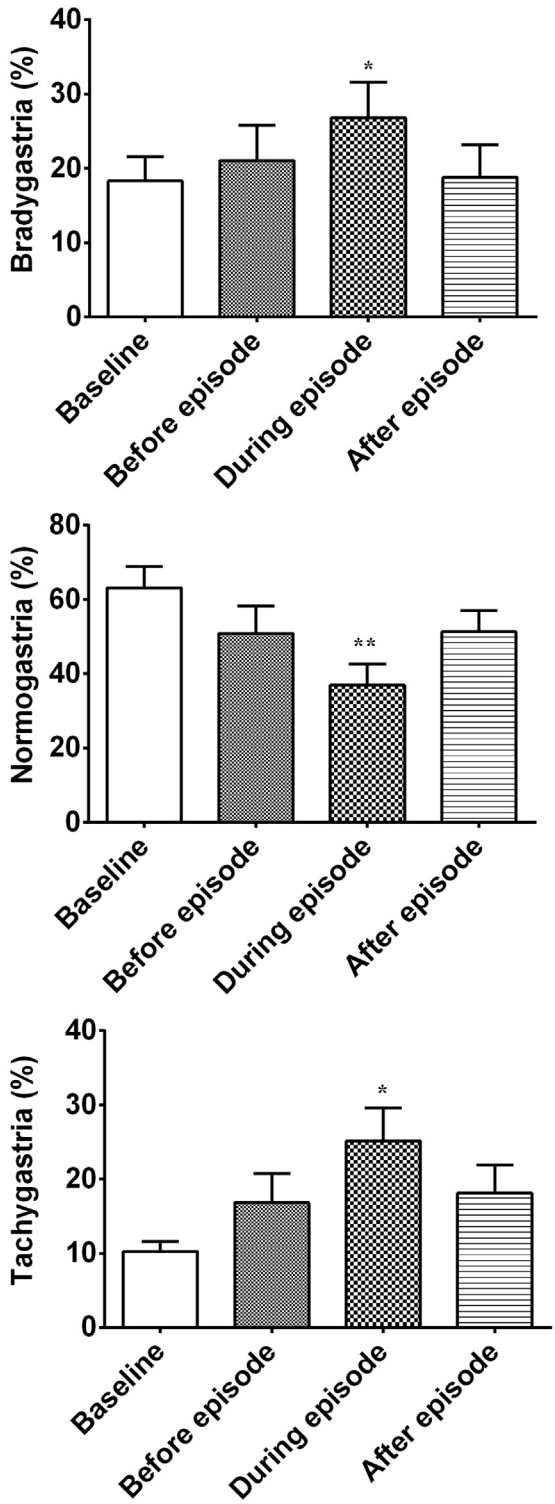

Fig. 5. Repartition (categorization into periods of bradygastria, normogastria, or tachygastria by power spectrum analysis - see Methods for details) of the GMA before the injection of exendin-4, immediately before emetic episodes, during emetic episodes and immediately after emetic episodes in the ferret. Ex-4 = exendin-4. Data represents the mean ± s.e.m. of 5 animals. Significant differences compared to baseline are shown as $*P < 0.05$, $**P < 0.01$ (One-way ANOVA followed by Bonferroni tests).

4.4. Nausea and vomiting

Our previous studies in ferrets have shown that i.c.v. administration of exendin-4 produces an inhibition of feeding and behaviours indicative of 'nausea' that is associated with an increase of c-Fos in the CeA, the bed nucleus of stria terminalis, cingulate cortex, and anterior (paraventricular nucleus, supraoptic nucleus) and tuberal (arcuate nucleus, dorsomedial and ventromedial nuclei) aspects of the hypothalamus. In animals also exhibiting emesis, there is also an additional increase in c-Fos activity in AP and NTS (Lu et al., 2016).

The present study extends our previous findings by showing that changes in GMA and HRV are associated with emesis in animals, and that these changes are considered causally linked to nausea in man (see Stern et al., 2011 for discussion). Our detailed analysis of GMA and HRV data suggests that the changes are mediated by an increase in sympathetic efferent drive, which is consistent with similar changes reported in humans experiencing nausea. Whilst it is not possible to know what the ferret is experiencing following exendin-4 administration, the changes in GMA and HRV, a decrease in food intake (present study), the pattern of behavioural changes indicative of nausea, and the pattern of brain c-Fos activity (Lu et al., 2016) are all consistent with the induction of nausea.

Centrally administered exendin-4 increases c-Fos activity in the brainstem, hypothalamus, and the limbic system (Lu et al., 2016). These brain regions have multiple functions, including induction and coordination of emesis that involves the AP and NTS, (Stern et al., 2011), rostral hypothalamus (Beleslin et al., 1987), an integration of autonomic outflow that involves the NTS, hypothalamus, CeA, cingulate cortex (Saper, 2002), mechanisms of food intake that involves the AP, NTS, dorsal and ventral medial hypothalamus, arcuate nucleus (Alhadeff and Grill, 2014; Baggio et al., 2008; Secher et al., 2014; Shirazi et al., 2013), responses to non-painful aversive stimuli including conditioned taste aversion that involves bed nucleus of the stria terminalis, CeA, cingulate cortex (Hayes and Northoff, 2012; Kinzig et al., 2003; St Andre and Reilly, 2007), and vasopressin release that involves supraoptic and paraventricular nucleus (Billig et al., 2001; Kim et al., 2011; Stern et al., 2011). The activation of a diverse range of brain regions is not unexpected when the range of behavioural and physiological responses (behaviours indicative of nausea, emesis, decreased food intake, hypertension, decreased HRV, GMA frequency/power) that can be affected by exendin-4 is considered, and that the brain regions concerned are inter-connected and the responses are related components of "nausea/visceral malaise" (see Lu et al., 2016).

4.5. Study limitations

Interpretation of the results from this telemetry study is limited by the use of a single dose of exendin-4, although as expected from our previous study (Lu et al., 2016), it evoked the full spectrum of expected effects on emesis and food intake and also revealed novel effects on GMA, HRV and temperature. Further studies using telemetry are required to investigate the dose-response relationships. Although limited, the present study together with the previous c-fos investigation of exendin-4 (Lu et al., 2016), provide enough information to illustrate the complexity of peripheral effects induced by central GLP-1 receptor activation.

Blood glucose levels following central exendin-4 were not measured. The effects of central exendin-4 on blood glucose (following a glucose load) in *Suncus murinus* are transient (< 1 h) (Chan et al., 2011), so even if central exendin-4 had a similar effect in this study we consider it is unlikely to account for the long lasting effects on GMA and HRV. Giving the ferrets a glucose load to investigate potential hypoglycaemic effects of exendin-4 would have confounded investigation of its effects on the GMA, as hyperglycaemia is known to modify gastric emptying and GMA (Stern et al., 2011). Conversely, in fasting ferrets blood glucose levels are relatively high (Fox, 2014), so it seems unlikely that central exendin-4 will make the animals clinically hypoglycaemic and hence induce the effects observed via autonomic activation. However, we are unable to exclude a contribution of transient reduction in blood glucose to the effects recorded and the

potential for central GLP-1 receptors to modulate blood glucose in a carnivore requires investigation.

5. Conclusion

The present study demonstrated that i.c.v. exendin-4 can induce changes in GMA and HRV that are comparable to those reported in humans reporting nausea and are consistent with being mediated by an increase in sympathetic dominance. These findings align with our previous demonstration that i.c.v. exendin-4 in the ferret induced behaviours indicative of nausea and the activation of c-Fos nuclei implicated in the modulation of autonomic outflow. The large and protracted (~24 h) hypertension in response to central exendin-4 is a novel finding in this species and requires further investigation and is relevant to the growing interest in the cardiovascular effect of GLP-1 receptor agonists (Drucker, 2016; Lorenz et al., 2017).

Conflict of interest

The authors have declared that no conflict of interest exists.

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