

## Reduced normogastric electrical activity associated with emesis: A telemetric study in ferrets

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### Abstract

**AIM:** To characterize the gastric myoelectric activity (GMA) and intra-abdominal pressure changes induced by emetic stimuli (apomorphine and cisplatin) in the ferret.

**METHODS:** GMA and intra-abdominal pressure were recorded in conscious, unrestrained ferrets surgically implanted with radiotelemetry transmitters. Animals were challenged with apomorphine (0.25 mg/kg *sc*) and cisplatin (10 mg/kg *ip*), and the emetic response was quantified *via* direct observation and intra-abdominal pressure recording for 1 and 4 h, respectively. The GMA was analyzed by spectral analysis; the parameters used to characterize the GMA were the dominant frequency (DF) and the repartition of spectral power in the bradygastric, normogastric and tachygastric frequency ranges.

**RESULTS:** Retches were identified on the intra-abdominal pressure trace as peaks  $0.30 \pm 1.01$  s in duration and  $59.57 \pm 2.74$  mmHg in amplitude, vomit peaks were longer ( $0.82 \pm 0.06$  s,  $P < 0.01$ ) and reached a higher pressure ( $87.73 \pm 8.12$  mmHg,  $P < 0.001$ ). The number of retches and vomits quantified

*via* direct observation [apomorphine:  $65.5 \pm 11.8$  retches + vomits (R+V), cisplatin:  $202.6 \pm 64.1$  R+V] and intra-abdominal pressure (apomorphine:  $68.3 \pm 13.7$  R+V,  $n = 8$ ; cisplatin:  $219.0 \pm 69.2$  R+V,  $n = 8$ ) were correlated ( $r = 0.97$ ,  $P < 0.0001$ ) and the timing of emesis was consistent between the 2 methods. Apomorphine induced a decrease in normogastria from  $45.48\% \pm 4.35\%$  to  $36.70 \pm 4.34\%$  ( $n = 8$ ,  $P < 0.05$ ) but the DF of the slow waves was not changed [ $8.95 \pm 0.25$  counts/min (cpm) *vs*  $8.68 \pm 0.35$  cpm,  $n = 8$ ,  $P > 0.05$ ]. Cisplatin induced a decrease in normogastria from  $55.83\% \pm 4.30\%$  to  $29.22\% \pm 5.16\%$  and an increase in bradygastria from  $14.28\% \pm 2.32\%$  to  $31.19\% \pm 8.33\%$  ( $n = 8$ ,  $P < 0.001$ ) but the DF ( $9.14 \pm 0.13$  cpm) remained unchanged ( $P > 0.05$ ). The GMA changes induced by cisplatin preceded the emetic response as normogastria was reduced for 1 h before the onset of emesis ( $57.61\% \pm 5.66\%$  to  $39.91\% \pm 5.74\%$ ,  $n = 6$ ,  $P < 0.05$ ). Peri-emesis analysis revealed that the GMA was significantly disturbed during and immediately after, but not immediately before, the emetic episodes.

**CONCLUSION:** The induction of emesis is reliably associated with a disrupted GMA, but changes may also occur prior to and following the emetic response.

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**Key words:** Emesis; Nausea; Stomach; Ferret; Cisplatin; Apomorphine; Electromyography

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### INTRODUCTION

Nausea and vomiting are components of the body's

defense response against toxins either ingested (e.g. plant alkaloids: nicotine, veratridine, emetine), or released following bacterial (e.g. *Staphylococcus aureus*, *Salmonella enterica*, *Vibrio cholerae*) or viral infection (e.g. norovirus, rotavirus)<sup>[1]</sup>. However, in the clinic, the emetic response can also be triggered inappropriately, for example, as a sequelae of anesthesia with surgery (postoperative nausea and vomiting)<sup>[2]</sup>, or as a side effect of cancer chemotherapy (e.g. cisplatin)<sup>[3]</sup>. In addition, nausea and vomiting are also commonly encountered during the pre-clinical and early clinical development of novel chemical entities (NCE) for a variety of therapeutic indications. Indeed, nausea and vomiting has been ranked second, after abuse liability, in the side effects negatively impacting on the development of NCE<sup>[4]</sup>.

Pre-clinical studies using species capable of emesis such as the ferret (*Mustela putorius furo* L.) or the least shrew (*Cryptotis parva*) correctly identified an emetic liability for several NCE. For example, emetic side effects were observed for phosphodiesterase-IV inhibitors<sup>[5]</sup>, a nicotinic receptor agonist<sup>[6]</sup>, and a cannabinoid CB1 receptor antagonist<sup>[7]</sup>, considered for the treatment of asthma, pain and obesity, respectively. Studies in the ferret also identified the anti-emetic potential of 5-hydroxytryptamine-3 (5-HT<sub>3</sub>) and tachykinin NK<sub>1</sub> receptor antagonists against cancer chemotherapy agents (see<sup>[8]</sup> for review), however, their more limited efficacy against nausea<sup>[9]</sup> could not be predicted from pre-clinical studies. Emesis can be divided into 2 components: the pre-ejection (or prodromal) phase and the ejection phase. The prodromal phase is characterized by sympathetic outputs such as cold sweating, skin vasoconstriction and pupil dilation. Additionally, under vagal control, the proximal stomach relaxes, delaying gastric emptying, and the retrograde giant contraction carries to the stomach intestinal contents. This phase is usually temporally correlated with the sensation of nausea<sup>[10]</sup>. Retching and emesis (vomiting), which constitute the ejection phase, are therefore end-stages in activation of the emetic reflex and there would be considerable utility in the identification of biomarkers induced at sub-emetic doses of a compound, which could be used to better identify the separation between a therapeutic dose and one with emetic liability (therapeutic index). Such biomarkers may provide insights into the potential for induction or detection and reduction of nausea, which remains controversial in studies using laboratory animals<sup>[4]</sup>.

In humans, correlates of nausea include an increase in plasma levels of vasopressin and changes in the electrogastrogram (EGG) frequency, rhythm and power<sup>[11]</sup>. The EGG reflects gastric myoelectric activity (GMA), or gastric slow waves; it is usually recorded from cutaneous abdominal electrodes to investigate the frequency of pacemaker activity, which underlies the genesis of gastric contractile activity<sup>[12]</sup>. However, the precise relationship of the EGG to motility itself is unclear<sup>[13]</sup> but tachygastria has been linked to gastric motor quiescence<sup>[14]</sup>. Of the 2 markers, only the recording of the gastric slow waves

provides a method amenable to telemetry with an adequate temporal resolution to examine relationships to emesis in unrestrained animals.

This paper reports the use of radiotelemetry in the conscious unrestrained ferret to record gastric slow wave activity and investigate the effect of the emetic challenges, apomorphine and cisplatin. Data are also included on telemetric recordings of intra-abdominal pressure following administration of emetic agents in this species. The results show that both apomorphine and cisplatin are associated with a reduction of normal gastric rhythm, and demonstrate the potential applicability of telemetric recording techniques to the study of emetic mechanisms and the identification and understanding of emetic liability of NCE. This provides insights into the changes in gastric function occurring prior to the onset of emesis and which, in humans, have been associated with the occurrence of nausea.

## MATERIALS AND METHODS

### Animals

Castrated male fitch (pigmented) ferrets (*Mustela putorius furo* L.) (1.17-1.65 kg) were obtained from Southland Ferrets (Invercargill, New Zealand). Prior to the experiments, they were housed communally in a temperature-controlled room at 24 ± 1°C, under artificial lighting with lights on between 06:00 am and 18:00 pm. Water and pelleted cat food (Tri Pro Feline Formula Cat Food, American Nutrition®, Utah, USA) were available *ad libitum* until the start of the experiments. All animals were then housed individually from the day of surgery to the end of the experiment. The experiments were conducted under the authority of a license provided by the Government of the Hong Kong SAR and approval from the Animal Experimentation Ethics Committee, The Chinese University of Hong Kong.

### Surgical techniques

**Telemetry transmitter implantation for GMA and abdominal pressure recording:** Anesthesia was induced with ketamine (20 mg/kg im; Alfasan, Holland) and maintained with isoflurane (Halocarbon Products Corporation, USA) about 1.5%, in a 3:1 O<sub>2</sub> to N<sub>2</sub>O ratio using a custom-made face mask and an anesthetic machine (Narkomed 2C, Drager, USA). Animals were placed on a heating pad (UCI#390 Analog moist heating pad, Rebirth Medical & Design, Inc., Taiwan) and the level of anesthesia was assessed and monitored throughout the surgery by the pedal withdrawal reflex. Following a midline abdominal incision, the antrum was exposed and the biopotential wires of the telemetry transmitter (C50-PTX, DSI, USA) were inserted in the muscle and secured in place by suturing the serosa. The body of the transmitter (with the pressure catheter) was inserted in the peritoneal cavity and sutured to the muscle layer on the side. The abdominal cavity was treated with antibiotic (Nebacetin®, Altana Pharma, Germany), sutured closed

in layers and covered with a permeable spray dressing (Opsite<sup>®</sup>, Smith and Nephew, UK).

**Analgesia and post-operative recovery:** Buprenorphine (0.05 mg/kg *sc*; Temgesic<sup>®</sup>, Schering Plough, UK) was given as a preoperative analgesic 15 min before the induction of anesthesia, and 12 h post surgery. Recovery was unremarkable and the wound healed within a week.

### Experimental design

Following surgery, animals were housed individually in observation cages (W49 cm × L61 cm × H49.5 cm). They were allowed to recover for at least 7 d prior to further experimentation. Some ferrets were administered apomorphine (0.25 mg/kg *sc*) at least 7 d prior to the administration of cisplatin (10 mg/kg *ip*). These doses of apomorphine and cisplatin have been shown to induce a reliable emetic response in the ferret<sup>[15,16]</sup>. At the end of the observation period, animals were killed with an overdose (> 100 mg/kg *ip*) of pentobarbital sodium (Dorminal<sup>®</sup>, Alfasan, Woerden, Holland).

Baseline telemetry recordings (GMA and abdominal pressure) were made for a least 1 h prior to presenting the animals with food, or an emetic challenge. Recordings then continued for 1 h in studies assessing the effect of food alone or apomorphine (0.25 mg/kg *sc*), or for 4 h in experiments assessing the action of cisplatin (10 mg/kg *ip*). Emesis was characterized by rhythmic abdominal contractions that were either associated with the oral expulsion of solid or liquid material from the gastrointestinal tract (i.e. vomiting), or not associated with the passage of material (i.e. retching movements). 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<sup>[17]</sup>. The latency was defined as the time between the administration of the drug and the first emetic episode.

**Effect of feeding on GMA:** Food was withdrawn for 12-14 h before the start of the studies. The ferrets were then presented with 20 g of pelleted cat food, and all uneaten food was withdrawn 10 min later. This design was chosen to mimic human studies describing the effect of a meal on GMA in humans<sup>[12]</sup>. GMA data were analyzed during the 5 min period prior to presentation of food, and during a 5 min post-prandial period, starting 5 min after the uneaten food was withdrawn.

**Effect of apomorphine (0.25 mg/kg *sc*) and cisplatin (10 mg/kg *ip*) on GMA and abdominal pressure:** On the day of the experiment water was freely available but food was withdrawn for approximate 2 h before the start of the studies. Ferrets were presented with pelleted cat food and 30 min later animals were injected subcutaneously with apomorphine (0.25 mg/kg) or saline (0.5 mL/kg NaCl 154 mmol/L); or injected intraperitoneally with cisplatin (10 mg/kg) or saline (10 mL/kg NaCl 154 mmol/L).

**Drugs:** Cisplatin [cis-diamminedichloroplatinum(II), David Bull Laboratories, Victoria, Australia] was purchased as a sterile saline solution at an active concentration of 1 mg/mL. Apomorphine hydrochloride (Sigma-Aldrich, St. Louis, USA) was dissolved in sodium metabisulphite (526 μmol/L, Riedel-de Haën, Germany) and injected at a concentration of 0.5 mg/mL and a volume of 0.5 mL/kg *sc*. Doses are expressed as the free base.

### Telemetry system and analysis of the data

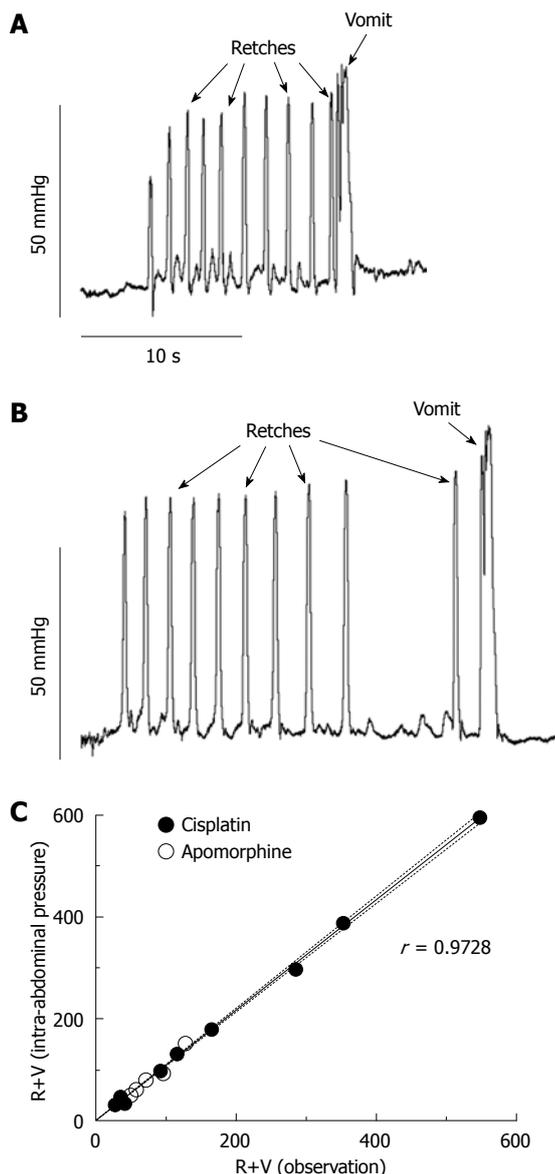
A DSI Dataquest<sup>®</sup> A.R.T. telemetry system (Data Science International, Minnesota, USA) was used. The GMA and intra-abdominal pressure were recorded using PhysioTel<sup>®</sup> C50-PXT Small Animal Transmitters. Telemetric signals were recorded *via* 2 receiver plates (PhysioTel<sup>®</sup> RPC-1) placed under the cages. The receivers were connected to a PC desktop computer *via* a matrix (Dataquest ART Data Exchange Matrix). An ambient pressure reference monitor (APR-1) was connected to the exchange matrix. Data was recorded with the Dataquest Acquisition software (DQ ART 4.0). Analysis of telemetry recordings was carried out using Spike2<sup>®</sup> (version 6.06, Cambridge Electronic Design, UK).

**Quantification of the retches and vomits *via* intra-abdominal pressure:** The abdominal pressure signal was recorded with a sampling frequency of 500 Hz. Retches and vomits were quantified from the intra-abdominal pressure recordings in a semi-automated manner. Thus, the traces of each ferret were inspected visually and then a detection threshold was set and pressure profiles corresponding to retches and vomits were isolated manually. To test the validity of this method, the number of retches + vomits (R+V) detected was compared to the number obtained *via* direct observation using a Spearman test for non-parametric correlation.

**GMA recordings:** The GMA signal was recorded with a sampling frequency of 1000 Hz; selected steps of the analysis procedure are presented in Figure 1. Briefly, a low pass finite impulse response (FIR) filter with a cut-off frequency of 2.5 Hz (transition gap: 10 Hz) was used to remove any signal with a frequency higher than 150 counts/min (cpm). The traces were then interpolated to a sampling frequency of 10.24 Hz and a second low pass FIR filter with a cut-off frequency of 0.3 Hz (18 cpm, transition gap: 0.1 Hz) was applied. This cut-off was chosen to filter out signals of probable cardiac (about 200 cpm in the ferret) and respiratory (33-36 cpm) origins<sup>[18]</sup>.

The following parameters were used to characterize the GMA (Figure 1): (1) the dominant power (DP, the highest power in the 0 to 15 cpm range); (2) the dominant frequency (DF, frequency bin with the highest power in the 0 to 15 cpm range); (3) the repartition of power in the bradygastric, normal and tachygastric ranges (i.e. bradygastria, normogastria and tachygastria). The DF





**Figure 2** Intra-abdominal pressure recordings. Intra-abdominal pressure was recorded via a telemetry transmitter in the abdominal cavity (C50-PTX, DSI®), showing emetic episodes—each composed of several retches and one vomit—induced by apomorphine (0.25 mg/kg sc) (A) and cisplatin (10 mg/kg ip) (B). Both recordings were obtained from the same animal. C: Correlation between the number of retches + vomits (R+V) induced by cisplatin (10 mg/kg ip) and apomorphine (0.25 mg/kg sc), quantified by direct observation and from the intra-abdominal pressure traces. Results plotted as individual values,  $n = 16$  (data obtained from 11 animals). Spearman correlation coefficient ( $r$ ), the linear regression (plain line) and 95% confidence interval (dotted line) are indicated on the graph.

increased by a ratio of  $6.80 \pm 3.61$ , to  $9.45 \times 10^{-4} \pm 2.84 \times 10^{-4}$  mV<sup>2</sup>. These changes were not statistically significant,  $P = 0.38$  and  $P = 0.13$  for the DF and DP respectively (paired  $t$ -tests,  $n = 10$ ).

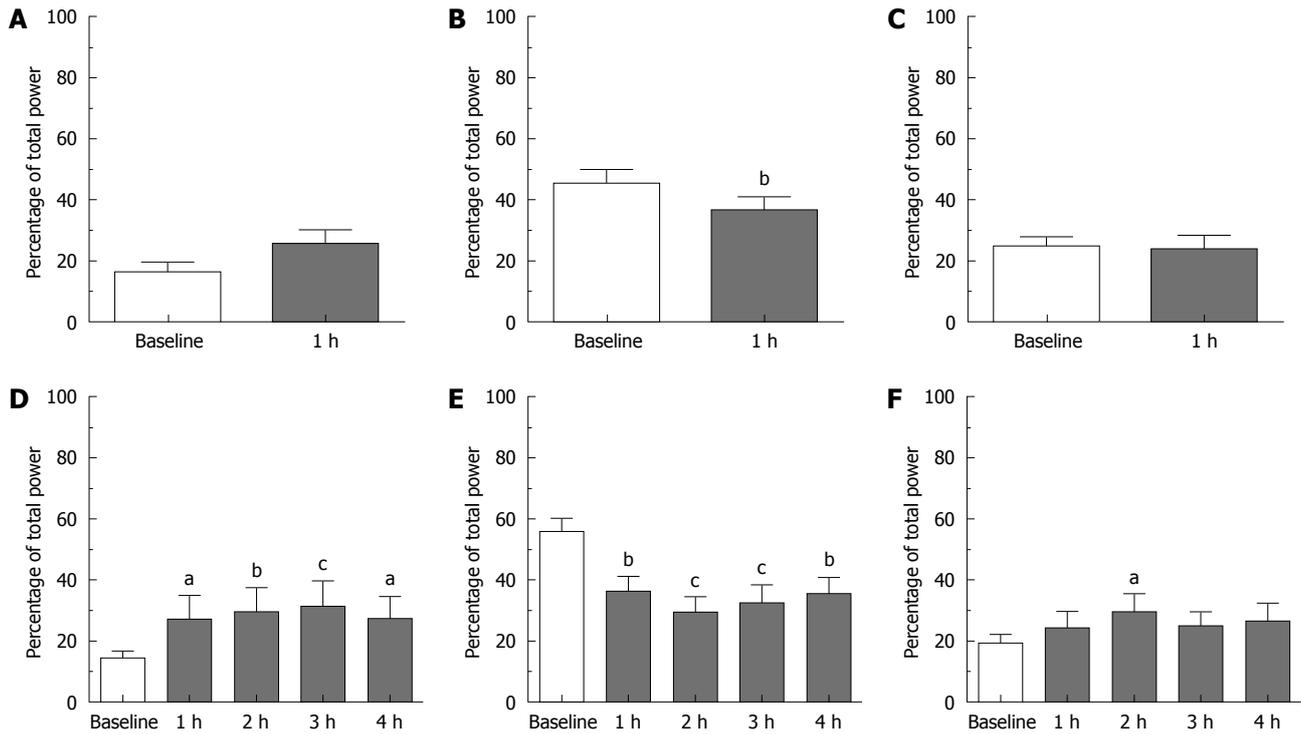
#### Effect of apomorphine and cisplatin on the GMA

**Effect of apomorphine:** During the 1 h that preceded the injection of saline (0.5 mL/kg sc), baseline bradygastria, normogastria and tachygastria were  $13.57\% \pm 3.53\%$ ,  $63.31\% \pm 9.99\%$  and  $16.26\% \pm 5.82\%$  of the total power, respectively. Baseline DF was  $9.54 \pm 0.26$  cpm

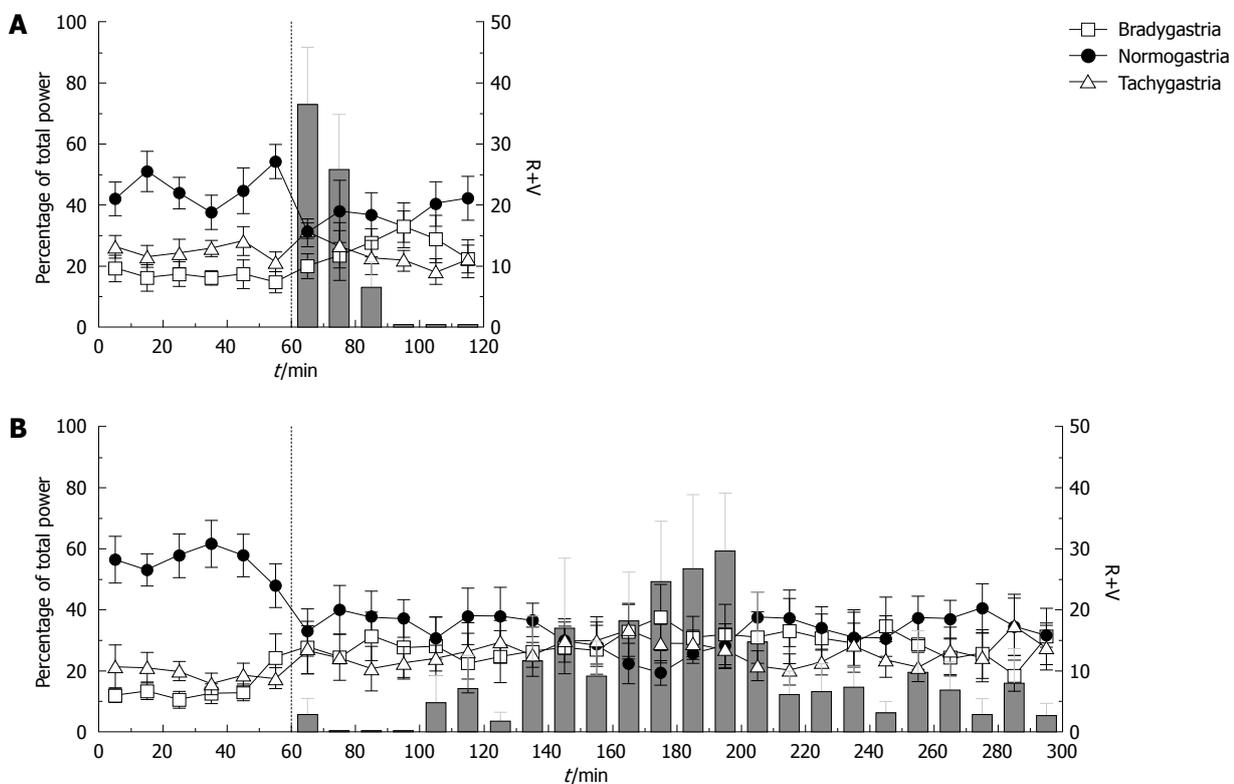
( $n = 4$ ). Following saline injection, the GMA repartition was  $15.14\% \pm 1.28\%$ ,  $57.40\% \pm 8.03\%$  and  $18.46\% \pm 6.13\%$  in the bradygastric, normal and tachygastria ranges respectively; the DF was  $9.73 \pm 0.24$  cpm. None of these values were significantly different from the baseline and no differences were detected between the saline and apomorphine groups during baseline ( $P > 0.05$ ). During the 1 h that preceded the injection of apomorphine, the GMA was repartitioned as follows: bradygastria:  $16.57\% \pm 3.17\%$ , normogastria:  $45.48\% \pm 4.35\%$  and tachygastria:  $25.66\% \pm 2.72\%$ ; DF:  $8.95 \pm 0.25$  cpm ( $n = 8$ ). As shown in Figures 3 and 4, following the administration of apomorphine, the percentage of power in the normal range decreased to  $36.70\% \pm 4.34\%$  ( $P < 0.01$ ), whereas the percentage of power in the bradygastric and the tachygastria ranges was not significantly altered:  $25.76\% \pm 4.65\%$  and  $22.77\% \pm 4.67\%$ , respectively ( $P > 0.05$ ). The DF did not change, and was  $8.68 \pm 0.35$  cpm following apomorphine [ $P > 0.05$ , two-way ANOVAs followed by Bonferroni post-tests,  $n = 8$  (apomorphine) and  $n = 4$  (saline)].

**Effect of cisplatin:** During the 1 h baseline recordings prior to the injection of saline (10 mL/kg ip), baseline bradygastria, normogastria and tachygastria were  $17.11\% \pm 5.35\%$ ,  $43.32\% \pm 6.37\%$  and  $25.85\% \pm 3.83\%$ , respectively; baseline DF was  $8.44 \pm 0.61$  cpm ( $n = 4$ ). The saline treatment had no significant effect on the GMA up to 3 h following intraperitoneal injection, however 4 h post injection the percentage of power in the tachygastria range was significantly increased compared to baseline to  $40.40\% \pm 8.31\%$  ( $P < 0.05$ ). During the 1 h baseline prior to the injection of cisplatin, the percentages in the bradygastric, normogastric and tachygastria ranges were  $14.28\% \pm 2.32\%$ ,  $55.83\% \pm 4.30\%$  and  $19.17\% \pm 3.08\%$ , respectively. The DF was  $9.14 \pm 0.13$  cpm ( $n = 8$ ). As shown in Figures 3 and 4, following the administration of cisplatin the percentage of power in the normogastric range decreased and reached a nadir in the second hour post-injection ( $29.22\% \pm 5.16\%$ ), whereas the percentage of bradygastria and tachygastria increased. Bradygastria reached a peak during the third hour after the injection ( $31.19\% \pm 8.33\%$ ) and tachygastria reached a peak during the second hour post-injection ( $29.56\% \pm 6.01\%$ ); the effects on normogastria and bradygastria were statistically significant during the entire observation period whereas the increase in tachygastria were only statistically significant 2 h post-injection ( $P < 0.05$ , Bonferroni post-tests compared to the 1 h baseline). The DF was not significantly altered after cisplatin administration ( $P < 0.05$ ). No differences could be detected at any time points between saline and cisplatin [ $P > 0.05$ , repeated measures two-way ANOVA followed by Bonferroni post-tests,  $n = 8$  (cisplatin),  $n = 4$  (saline)].

A secondary analysis was carried out only on the animals with a latency to the onset of emesis greater than 1 h ( $n = 6$ ). In these animals, the percentage of power in the normogastric range was significantly reduced in



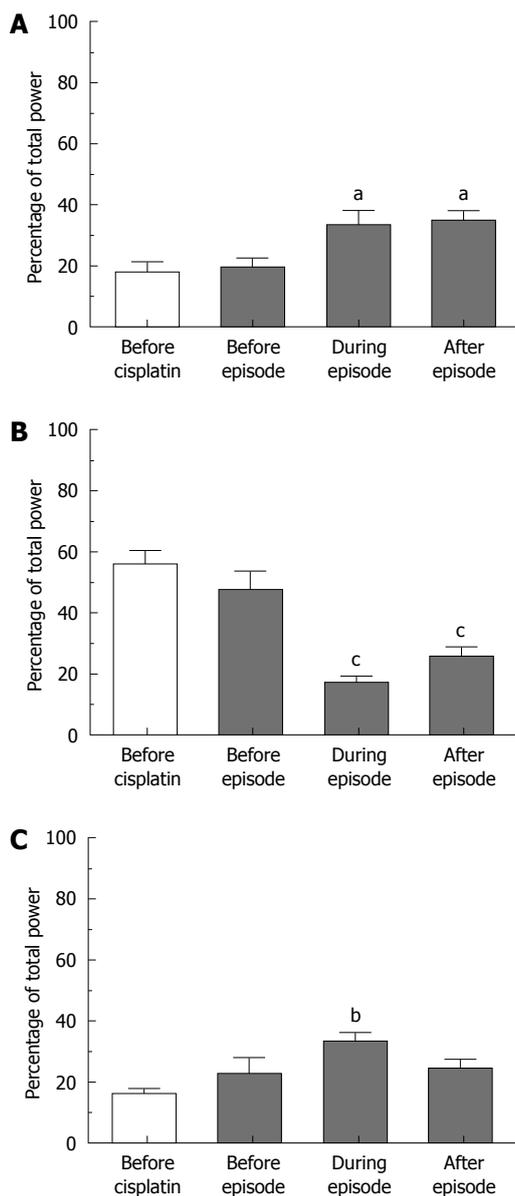
**Figure 3** Effect of apomorphine (0.25 mg/kg sc, A-C) and cisplatin (10 mg/kg ip, D-F) on the gastric myoelectric activity (GMA) in ferrets. The graphs represent the percentage of total (0-18 cpm) power in the bradygastric range [0 - (DF - 1 cpm), A and D], in the normogastric range [(DF - 1 cpm) - (DF + 1 cpm), B and E] and in the tachygastric range [(DF + 1 cpm) - 15 cpm, C and F]. Results are plotted as mean ± SE (n = 8). Differences compared to the effect of a dose of saline (sc or ip as appropriate, data not shown on this graph) were computed using two-way ANOVA (factors: treatment and time) and Bonferroni post-tests. Differences with baseline are indicated as <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.001.



**Figure 4** Profile of emesis and GMA repartition in the bradygastric, normogastric and tachygastric ranges following the administration of apomorphine (0.25 mg/kg sc) (A) and cisplatin (10 mg/kg ip) (B) in the ferret. Data plotted as mean ± SE per 10 min, n = 8.

the first hour post-cisplatin injection from 57.61% ± 5.66% to 39.91% ± 5.74% (P < 0.05) even though no

emesis was observed during that period. Percentages of power in the bradygastric and tachygastric ranges



**Figure 5** Repartition of the GMA in the bradygastric (A), normogastric (B) and tachygastric (C) ranges before the injection of cisplatin, immediately before emetic episodes, during emetic episodes and immediately after emetic episodes induced by cisplatin (10 mg/kg ip) in the ferret. Results are plotted as mean  $\pm$  SE,  $n = 8$ . Differences compared to baseline are calculated using repetitive measures one-way ANOVA followed by Bonferroni post tests. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$ .

were unchanged [ $P > 0.05$ , repeated measures two-way ANOVA followed by Bonferroni post-tests,  $n = 6$  (cisplatin),  $n = 4$  (saline)].

We investigated the GMA repartition surrounding and during emetic episodes; altogether, 40, 41, 62 and 41 determinations were used to characterize the GMA repartition before cisplatin and before, during and after emetic episodes, respectively. Bradygastric, normogastric and tachygastric values during the 10 min preceding the injection of cisplatin were  $17.67\% \pm 3.50\%$ ,  $56.00\% \pm 4.41\%$  and  $16.15\% \pm 1.66\%$  (mean  $\pm$  SE for 8 animals), respectively. Peri-emesis, the percentage repartition in all 3 ranges was significantly altered ( $P = 0.0085$ ,  $P < 0.0001$  and  $P = 0.0261$  for bradygastric, normogastric

and tachygastric respectively, repeated measures one-way ANOVAs, Figure 5). Bonferroni post-tests revealed that these values did not change significantly immediately before an episode (bradygastric:  $19.36\% \pm 3.02\%$ , normogastric:  $47.56\% \pm 6.18\%$  and tachygastric:  $22.87\% \pm 5.20\%$ ,  $P > 0.05$ ), but were altered during (bradygastric:  $33.08\% \pm 4.90\%$ , normogastric:  $17.23\% \pm 2.06\%$  and tachygastric:  $33.27\% \pm 3.00\%$ ,  $P < 0.05$ ) and immediately after emetic episodes (bradygastric:  $34.68\% \pm 3.33\%$ , normogastric:  $25.57\% \pm 3.27\%$  and tachygastric:  $24.38\% \pm 3.12\%$ ,  $P < 0.05$ ).

## DISCUSSION

### Recording and analysis of GMA in the ferret

The present study is the first report of ambulatory gastric myoelectric recordings in conscious unrestrained ferrets. The frequency of the gastric slow waves was  $9.12 \pm 0.23$  cpm (mean  $\pm$  SE of 12 animals), which is in accordance with post-prandial frequency of antral contractions ( $8.8 \pm 0.5$  cpm)<sup>[19]</sup>, and the frequency of the antral slow waves (9.5 cpm) reported by Diamant *et al.*<sup>[20]</sup> in abstract form; both were measured in conscious but restrained ferrets. In contrast to common protocols used to investigate the GMA in humans<sup>[21-23]</sup> and other animals (dog)<sup>[24,25]</sup>, which used a fixed normogastric range (typically 2.5-3 to 3.7-4 cpm in humans and 4-6 cpm in dogs)<sup>[12,26,27]</sup>, normogastric was defined according to each animal's intrinsic slow waves frequency during baseline. This was done for 2 reasons: (1) because of a relative paucity of literature, not enough data on the ferret's GMA was available to determine with confidence what the normogastric range should be (DF range 7.8-9.2 cpm in 12 animals in the present study) or how wide it should be; (2) the purpose of the present study was to focus on the changes induced by an experimental stimulus; setting the normogastric range relative to each individual animal reduced the influence of inter-animal variability and rendered the analysis more powerful to detect the effect of an emetic stimulus on the GMA in a limited number of animals.

Regarding the GMA analysis parameters, we chose to report the DF and compute the repartition of power in the normogastric, bradygastric and tachygastric ranges. An alternative analysis, which has been used in most preclinical studies<sup>[27-29]</sup> and some studies in humans<sup>[30]</sup>, consists of calculating the percentage of time during which the DF falls within each frequency range. The analysis parameters used in the present study were chosen as the power repartition encompass all the data present in the GMA signal and does not solely focus on the time at which the DF is included within a range of interest<sup>[12]</sup>.

### GMA changes induced by apomorphine and cisplatin

Similar GMA changes were observed following apomorphine and cisplatin; both stimuli induced a decrease in the percentage of power in the normogastric range, which was accounted for in the case of cisplatin by a clear increase in power in the bradygastric range,

without changing the DF. Our findings on the effects of apomorphine are partially supported by a number of studies in conscious, restrained dogs, which also reported a transient disruption of the gastric antral rhythm following the administration of apomorphine<sup>[31-33]</sup>. To the best of our knowledge, the present study represents the first clear evidence that apomorphine is associated with a reduction of normogastria for up to 1 h, which outlasts the emetic response as the last emetic episode was observed  $21.12 \pm 1.76$  min ( $n = 8$ ) post-apomorphine.

Regarding the effect of cisplatin, GMA changes were temporally correlated with the occurrence of emesis and maximal changes in the GMA repartition were observed when the emetic response was the most intense (2-3 h post cisplatin). However, GMA changes preceded the emetic response as evidenced by a decrease in normogastria during the first hour post-cisplatin in animals, which had not yet developed emesis during that period. Consistent with our findings, a recent study in the dog showed that cisplatin reduced the percentage of normal gastric slow waves in the hours preceding the onset of emesis and during the emetic response<sup>[34]</sup>. In the present study, a peri-emesis analysis revealed that normogastria was decreased and bradygastria and tachygastria were increased during and immediately after-but not immediately before-an emetic episode, which is in accordance with the short periods of dysrhythmia associated with emetic episodes have been reported in human patients during the administration of cisplatin<sup>[35]</sup>. Our findings are partly supported by human studies, which reported dysrhythmias in a few patients treated with anti-cancer chemotherapy. However, such events appeared to be transient rather than an overall change in slow wave activity<sup>[30,35]</sup>. The apparent differences in the effect of chemotherapy in human patients and in the ferret model could have four explanations:

(1) In the present study, the ferret model of acute cisplatin-induced emesis was used. This model uses a high bolus dose of cisplatin (10 mg/kg ip)-a highly emetic chemotherapeutic agent-to provoke an intense, reliable emetic response in all the animals, typically quantifiable over 4 h<sup>[36]</sup>. In human patients however, chemotherapy (platinum-based or not) is infused over hours and the emetic response is less reliable. Correspondingly, the GMA disturbance may be more severe in the ferret model than it is in human patients.

(2) Additionally, cancer patients treated with chemotherapy are not healthy subjects and their GMA may already be altered before they receive chemotherapy, rendering it less likely to detect an additional effect of the anti-cancer treatment. It is interesting that following a chemotherapy session, Riezzo *et al.*<sup>[30]</sup> reported a higher percentage of tachygastria in cancer patients compared to healthy volunteers. However, they did not compare the EGG repartition in the cancer patients prior to chemotherapy, the measurements were collected 7 d post-chemotherapy and the chemotherapy regimens were not reported, precluding any direct comparison with the present study.

(3) The use of anti-emetic prophylaxis may also have an influence on GMA, and 5-HT<sub>3</sub> receptor antagonists-commonly administered with chemotherapy and used in the 2 above-mentioned human studies-have been reported to reduce vection-induced dysrhythmias<sup>[37]</sup>.

(4) The analysis of the data is an important factor to consider; as discussed above, in the present study, the DF and the percentage repartition of the power in the bradygastric, tachygastria and normogastria ranges were computed. In contrast, Samsom *et al.*<sup>[35]</sup> identified bradygastria and tachygastria as intervals of at least 2 min, during which the DF was  $< 2.4$  cpm or  $> 3.6$  cpm and no overall assessment of normogastria was made, apart from the mean DF. Also, Riezzo *et al.*<sup>[30]</sup> calculated the percentage of successive spectra in which the DF falls within one of the 3 ranges, or used visual inspection of the waveform traces or the regular spiking activity (RSA).

### **Intra-abdominal pressure recordings**

On the intra-abdominal pressure traces, retches were identified as brief peaks whereas vomits were more prolonged. These findings in the freely moving, conscious ferret are consistent with what McCarthy *et al.*<sup>[38]</sup> described in the decerebrate cat. Our technique represents a great advantage in that quantifying the retches and vomits from the intra-abdominal pressure traces is more accurate than from a video or even direct observation; the distinction between retches and vomits is unequivocal and abdominal pressure recordings enable precise analysis regarding the timing and the frequency of the retches. Further information regarding the central neuronal circuitry involved in the mechanism of various emetics and anti-emetics can be gained from, for example, the interval between retches, the pattern of retching preceding a vomit or the characteristics of episodes including a vomit and those of episodes of retches. The major disadvantage is that this is an invasive technique, which requires abdominal surgery.

## **CONCLUSION**

The use of telemetry enables the recording of gastric slow waves in freely moving, conscious animals; additionally, analysis of the emetic response *via* intra-abdominal pressure permits a more rigorous data collection and the investigation of the precise temporal correlation between gastric changes and emesis. Using an EGG-like analysis of GMA recordings, disruption of the gastric rhythm was detected in ferrets challenged with apomorphine and cisplatin. Both emetic stimuli were associated with a reduction of normogastria, and cisplatin increased bradygastria. The GMA changes were subtle and not detectable by a simple DF analysis but comparison of the power repartition of the gastric signal, before and after the emetic challenges, indicated a clear change, which was temporally correlated with the development of the emetic response but not restricted to the immediate peri-emetic period. In the ferret, recording and analysis of the GMA, which is a physiological correlate of nausea in

humans, will improve the understanding of the changes in gastric function associated with emesis.

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## COMMENTS

### Background

Nausea is a subjective sensation, therefore impossible to study directly in animals. However physiological correlates of nausea have been identified in humans and include disruption of the gastric electrical rhythm. As nausea usually precedes the onset of emesis, recording the gastric electrical rhythm in animals potentially enables the detection of emetic pathway activation before the emetic threshold is reached.

### Research frontiers

The application of human biomarkers of nausea to an animal model enables the refinement of this model as it improves its translational potential. The research hotspot is the correlation of physiological events occurring around the time of vomiting, which may give insight into nausea.

### Innovations and breakthroughs

Recent *in vivo* research studies investigating the relationship between emesis and gastric myoelectric activity (GMA) have used either restrained or anesthetized animals (mainly dogs) with wires connected to the serosa and externalized through the skin. The use of telemetry represents a novel approach and the present study is the first report of GMA recording in freely moving animals, which represents an indisputable advantage in terms of animal welfare.

### Applications

The ferret is a species commonly used in emesis research and our approach could be integrated to standard study designs, therefore refining this animal model by enabling the detection of emetic pathway(s) activation before the emetic threshold is reached.

### Terminology

The GMA consists of electrical pacemaker signals, which trigger contractions of the stomach. Gastric dysrhythmia refers to a departure from the normal gastric electrical rhythm, the term encompasses bradygastria, tachygastria and gastric arrhythmia.

### Peer review

This is a well written manuscript from an established group expert in this field using a novel relevant approach to assess GMA and intra-abdominal pressure in the conscious ferret implanted with radiotelemetry transmitters.

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