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ORIGINAL ARTICLE

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Ghrelin alleviates cancer chemotherapy-associated dyspepsia in rodents

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Abstract *Purpose*: Chemotherapy treatment may lead to delayed gastric emptying, early satiety, anorexia, nausea and vomiting, described collectively as the cancer-associated dyspepsia syndrome (CADS). *Method*: We examined the effects of ghrelin in rodent models of CADS induced by treatment with cisplatin. *Results*: In rats, increased gastric contents and reduced feeding were observed 48 h after injection with cisplatin (6 mg/kg, i.p.). Ghrelin (0.5 mg/kg, i.p.) caused a 16-fold increase in food intake over 1 h in cisplatin/ghrelin-treated rats compared to cisplatin/vehicle-treated rats. A single dose of ghrelin also restored the decreased locomotor activity in rats induced by cisplatin to almost the same level of saline-treated rats. In mice, daily food intake was significantly decreased at 24 h (60%) and 48 h (74%) after cisplatin (20 mg/kg, i.p.). Ghrelin (1 mg/kg, i.p.×2) significantly increased food intake measured at the 48 h time-point in both saline/ghrelin-treated and cisplatin/ghrelin-treated mice, with this effect being most marked in the cisplatin-treated group, where a twofold increase in feeding was observed. In cisplatin-treated mice, delayed gastric emptying was indicated by a 7.7-fold increase in the wet weight of gastric contents and ghrelin improved the gastric emptying index (GEI) by 31% ($P < 0.01$). *Conclusion*: Together, these results suggest that it is possible to model cancer chemotherapy-induced dyspepsia in rodents and that ghrelin can greatly alleviate the behaviours associated with this syndrome. Agonists at the ghrelin receptor may, therefore, become a useful human therapeutic for this disorder.

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Introduction

Anti-cancer chemotherapy is associated with a number of undesirable side-effects which include acute and delayed nausea and vomiting [21, 42], reduced food intake [20], decreased body weight and disrupted gastrointestinal function [26]. Delayed gastric emptying has been suggested recently as a common cause of these gastrointestinal symptoms, which can also be a complication of the disease itself as well as its treatment. This cluster of symptoms has been referred to as cancer associated dyspepsia syndrome (CADS) with gastric prokinetic agents being the mainstay of treatment [36, 37].

Ghrelin is a 28-amino acid peptide found predominantly in the stomach, acting as the endogenous ligand for the ghrelin or growth hormone secretagogue receptor see Refs. [13, 25, 40]. There is an extensive literature demonstrating that ghrelin can act within the hypothalamic arcuate nucleus, pituitary and elsewhere (e.g., vagus), to regulate growth hormone secretion, food intake and energy balance e.g., Refs. [4, 12, 22, 23, 30, 44, 50]. However, a major new role for ghrelin is increasingly becoming recognised, namely that of regulating and protecting the upper gastrointestinal tract [6, 46]. Functional ghrelin receptors have been located within the enteric nervous systems of rats and humans [10, 15], and on the rat vagus nerve [3, 11] where nerve fibres projecting to the stomach have been shown to express ghrelin receptors [45]. Together, these systems have been implicated in the control of food intake by rodents [3], the facilitation of gastric contractions in anaesthetised rats [32], the induction of fasted gastrointestinal motor activity in conscious rats [15, 18], the acceleration of gastric emptying in mice or rats [3, 32] and improvements in post operative gastric ileus in dogs [48] and rats [47] or septic ileus in mice [14].

Recently, in cancer patients with impaired appetite, ghrelin has been shown to increase energy intake and meal appreciation during a buffet meal [35]. In order to further investigate the role for ghrelin in the management of cancer chemotherapy-associated dyspepsia and perhaps other forms of dyspepsia, we established rat and mouse models of gastric stasis using cisplatin, a broad-spectrum anticancer drug clearly associated with the induction of emesis and dyspepsia in cancer patients; the use of both species minimised the possibility of measuring species-specific responses, an important consideration given the ability of rats but not mice to exhibit pica in response to cisplatin [29]. In these experiments, we also measured changes in feeding and locomotor behaviours as well as the gastric stasis [31]. From these data we were then able to make comment on the ability of ghrelin to affect multiple parameters of dyspepsia, measured simultaneously in the same animal and in two different species.

Materials and methods

Animals

Adult male Wistar rats and C57/*6J* black mice were obtained from A. Tuck & Son Ltd. (Essex, UK). They were maintained on a conventional pelleted stock diet (Bantin and Kingman, Hull, UK) with free access to water in humidity and temperature-controlled rooms ($21 \pm 1°C$) with a 12-h light/dark cycle (lights on 0700 h). All experiments were performed under the UK Animal (Scientific Procedures) Act 1986.

Drugs

Rat ghrelin was purchased from BACHEM Ltd. (Merseyside, UK) and was dissolved in 0.01% BSA/saline. Cisplatin (cis-platinum II-diammine dichloride; Sigma, Dorset, UK) was dissolved in sterile saline.

Protocols

Study 1: effect of a single dose of ghrelin on food intake in rat treated with cisplatin for 48 h

Rats were housed individually 3 days prior to the start of the experiment. They were randomised into four treatment groups: saline/vehicle, saline/ghrelin, cisplatin/vehicle, cisplatin/ghrelin. On the test day, two groups of the rats received cisplatin (6 mg/kg, i.p.) at 11.00 am, and the remaining two groups were given saline (4 ml/kg, i.p.); this dose of cisplatin was previously shown to be well tolerated by rats. Body weight, food and water consumption were monitored daily at between 9.30 am and 10.00 am. On day 2 (48 h post cisplatin administration), a single dose of ghrelin was given (0.5 mg/kg, i.p.) at 11.00 am to investigate the acute effects on ghrelin. Food intake was measured 1 h after ghrelin injection and the experiment was then terminated. We chose to administer ghrelin at this time because previous experiments demonstrated that rats developed gastric stasis 2 days after administration of cisplatin 6 mg/kg i.p., at which time feeding was also reduced [29]. However, after 3 days, food intake started to recover. Accordingly, we have investigated the effects of acute administration of ghrelin on food intake and gastric contents at a time when the effects of cisplatin on gastric function may be most marked. This study was intended to identify the potential of ghrelin for treatment of disordered gastric function and food intake once established. At the end of the experiment rats were killed by using a rising concentration of CO_2 and cervical dislocation (Schedule 1 method); gastric contents were then removed and weighed (wet weight).

Study 2: effect of a single dose of ghrelin on activity in rat treated with cisplatin for 48 h

A photobeam activity system (AM1053 Amlogger; Linton Instruments, Norfolk, UK) was used to monitor activity [31]. Rats were placed in individual clear plastic cages surrounded by an infrared array. This array consists of two levels of infrared beams (3.0 and 11.0 cm from the bottom of the cage) with 24 on each level arranged in an 8×16, 25.4 mm pitched grid. Activity was measured as number of seconds where at least one beam was broken on the lower level (horizontal movement). Mobility (as measure for locomotion) was the time spent moving at least 50 mm across the lower level. The upper level beams were for the measurement of rearing. After a 3-day adaptation period in their activity cage, rats were randomised into three groups on the test day: saline/vehicle, cisplatin/vehicle, cisplatin/ghrelin. Two groups of rats were given cisplatin (6 mg/kg, i.p.) and the other group received saline (4 ml/kg, i.p.) at 11.00 am., and activity was measured continuously. A single dose of ghrelin (0.5 mg/kg, i.p.) was administered at 11.00 am 48 h after cisplatin and saline injection. Activity data was harvested from a 4-h period (11.00–15.00 h) at 24 and 48 h post cisplatin treatment. The rationale for this single dose study is as described in Study 1 above. At the end of the experiment rats were killed by using a rising concentration of CO_2 and cervical dislocation (Schedule 1 method).

Study 3: effect of 2-day ghrelin treatment on daily food and water consumption, body weight, gastric content in mice treated with cisplatin

Mice were housed singly 3 days prior to the start of the experiment. On the test day, they were randomised into four treatment groups: saline/vehicle, saline/ghrelin, cisplatin/vehicle and cisplatin/ghrelin. Body weight, food and water were recorded at between 9.30 am and

10.00 am. Ghrelin (1 mg/kg) or vehicle (5 ml/kg) was administered intraperitoneally (i.p.) at 10 am. The mice were then given either cisplatin (20 mg/kg; i.p.) or saline (5 ml/kg) 45 min after the first dose of ghrelin; this dose of cisplatin was previously shown to be well-tolerated by mice [29]. A second dose of ghrelin (1 mg/kg, i.p.) was then given at 5.00 pm. The same dose of ghrelin was administered again (at 10 am and 5 pm) 24 h post cisplatin treatment (day 1). Body weight, food and water intake were recorded daily at between 9.30 am and 10 am. The experiment was terminated on day 2 (48 h post cisplatin treatment). Mice were killed by using a rising concentration of CO_2 and cervical dislocation (Schedule 1 method); gastric contents were then removed and weighed (wet weight). The effects of ghrelin treatment on gastric emptying rate were then calculated as: gastric emptying index (GEI) % = [1−(weight of stomach content ÷ food consumed between 24 and 48 h post cisplatin)]×100.

Statistics

Results are presented as mean ± SEM. Statistical comparisons of the different treatment groups were made by one-way analysis of variance followed by Dunnett's multiple comparisons test. All probabilities quoted are two-tailed, with $P < 0.05$ being taken as the level of significance.

Results

Effect of a single dose of ghrelin on food intake in cisplatin-treated rats

Two days after cisplatin injection (6 mg/kg, i.p.), stomach contents were collected and weighed. The wet weight of the contents was 4.8-fold higher in cisplatin-treated, compared to saline-treated rats (cisplatin: 8.51 ± 1.1 g; saline: 2.02 ± 0.60 g; $n = 6$; $P < 0.01$). In separate groups of rats, both saline- and cisplatin-treated rats were given either ghrelin (0.5 mg/kg, i.p.) or vehicle 48 h post cisplatin treatment. Food intake was measured 30 min before ghrelin injection and then 1 h thereafter. A single dose of ghrelin significantly increased food intake over this 1 h period in the saline/ghrelin-treated rats, compared to that of the saline/vehicle-treated rats (3.1-fold, $P < 0.05$; Fig. 1a). A 16-fold increase was seen in the cisplatin/ghrelin-treated group compared to the cisplatin/vehicle-treated rats ($P < 0.001$, Fig. 1a). When the food consumed over the 1 h period following ghrelin treatment was expressed as a percentage of the food intake during the last 24 h period (24–48 h post cisplatin) prior to ghrelin administration, a 34% increase was found in the cisplatin/ghrelin-treated animals compared to a 2.3% increase in cisplatin/vehicle-treated, and 7.1% increase in the saline/ghrelin-treated groups ($P < 0.01$ for each comparison; Fig. 1b).

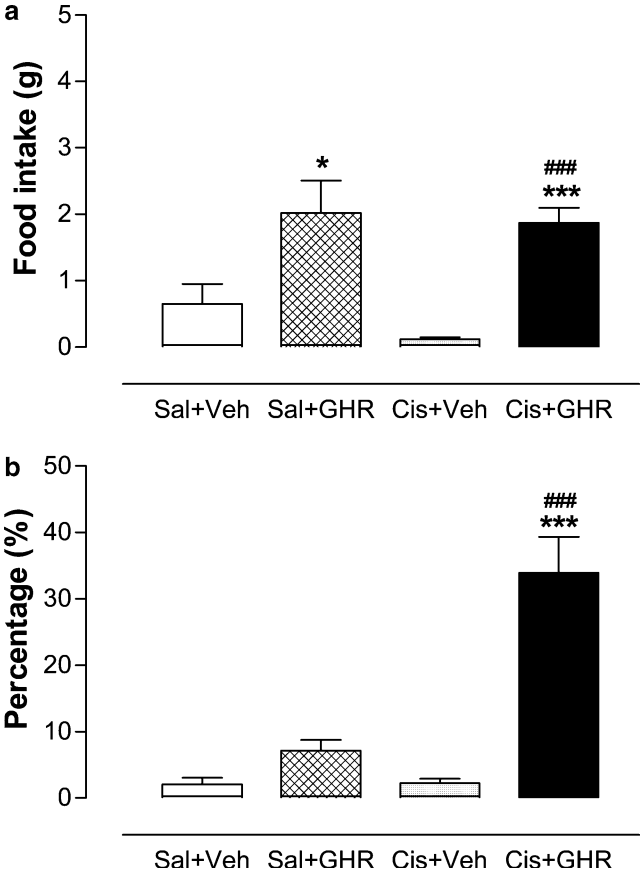

Fig. 1 Food intake after a single dose of ghrelin (0.5 mg/kg, i.p.) in rats treated with cisplatin (6 mg/kg, i.p.) for 48 h. **a** 1 h food consumption after ghrelin; **b** 1 h food consumption as percentage food intake of 24 h period (24–48 h post cisplatin) prior to ghrelin administration. Results are mean ± SEM ($n = 5$–10). $^{*}P < 0.05$; $^{***}P < 0.001$ vs. saline + vehicle; $^{\#\#\#}P < 0.001$ vs. cisplatin + vehicle (Dunnett's test)

Effects of ghrelin on locomotor activity in rats

Mean horizontal movement (time spent breaking at least one beam over a 4 h observation period, 11.00–15.00 h) was significantly decreased (by 38%) 24 h after cisplatin administration (6 mg/kg, i.p. at 11.00 h; Fig. 2). A single dose of ghrelin (0.5 mg/kg, i.p.), given 48 h after cisplatin injection at 11.00 h, increased the mean activity during the next 4 h period by 66% in the cisplatin/ghrelin-treated rats, compared to the cisplatin/vehicle-treated animals where there were no significant changes in activity over this time period. Further, there was no statistically significant difference between the saline/vehicle-treated and cisplatin/ghrelin-treated animals (Fig. 2). A single dose of ghrelin (0.5 mg/kg, i.p.) was without significant effect on activity over a 4 h period in animals previously treated with saline alone (control 34.5 ± 4.3 min spent breaking at least one beam versus ghrelin treated 35.5 ± 5.2 min spent breaking one beam, $n = 5$). The total activity was not significantly different from that in the cisplatin/ghrelin group but was signifi-

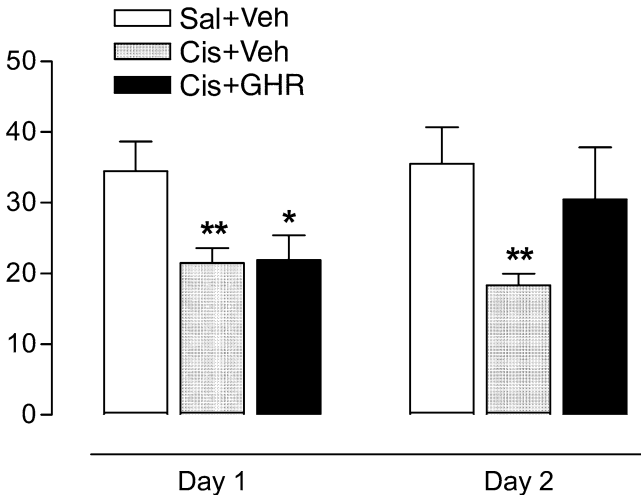

Fig. 2 Effect of a single dose of ghrelin (0.5 mg/kg, i.p.) on activity (4 h mean horizontal movement, 11.00–15.00 h) in rats treated with cisplatin (6 mg/kg, i.p.) for 48 h. Results are mean ± SEM ($n = 5$–10). *$P < 0.05$; **$P < 0.001$ vs. saline + vehicle (Dunnett's test)

cantly different from the cisplatin/saline group ($P < 0.01$).

Effects of 2-day ghrelin treatment on food, water intake and body weight in mice

Daily food intake was significantly decreased by cisplatin treatment (20 mg/kg, i.p.), with 60 and 74% reductions observed, respectively, 24 and 48 h after cisplatin dosing; $P < 0.01$ vs. saline, Fig. 3a). Ghrelin administration (1 mg/kg, i.p., twice daily) tended to increase food intake 24 h after dosing with saline or cisplatin (by 24 and 12%, respectively; non-significant). However, 48 h post cisplatin, food intake was significantly increased in saline/ghrelin-treated mice (by 38%, $P < 0.01$ vs. saline/vehicle) and a much larger, twofold increase in food intake was observed in the cisplatin/ghrelin-treated group ($P < 0.01$ vs. cisplatin/vehicle; Fig. 3a). Daily water consumption was significantly decreased after 24 or 48 h in the cisplatin-treated animals, compared to that of the saline-treated mice (35 and 57%, respectively; $P < 0.01$). Ghrelin administration had no significant effect on water consumption in either saline- or cisplatin-treated animals (Fig. 3b). A small weight loss was seen in the saline-treated mice 24 (−0.23 g) and 48 h (−0.30 g). During cisplatin treatment there was a further weight reduction at 24 and 48 h post administration (−2.0 and −1.4 g, respectively; $P < 0.01$, Fig. 3c). By contrast, body weight tended to increase in saline-treated animals 24 or 48 h after ghrelin administration and there was a trend for less reduction in body weight in the cisplatin-treated mice 24 h after ghrelin administration; neither of these trends reached statistical significance (Fig. 3c).

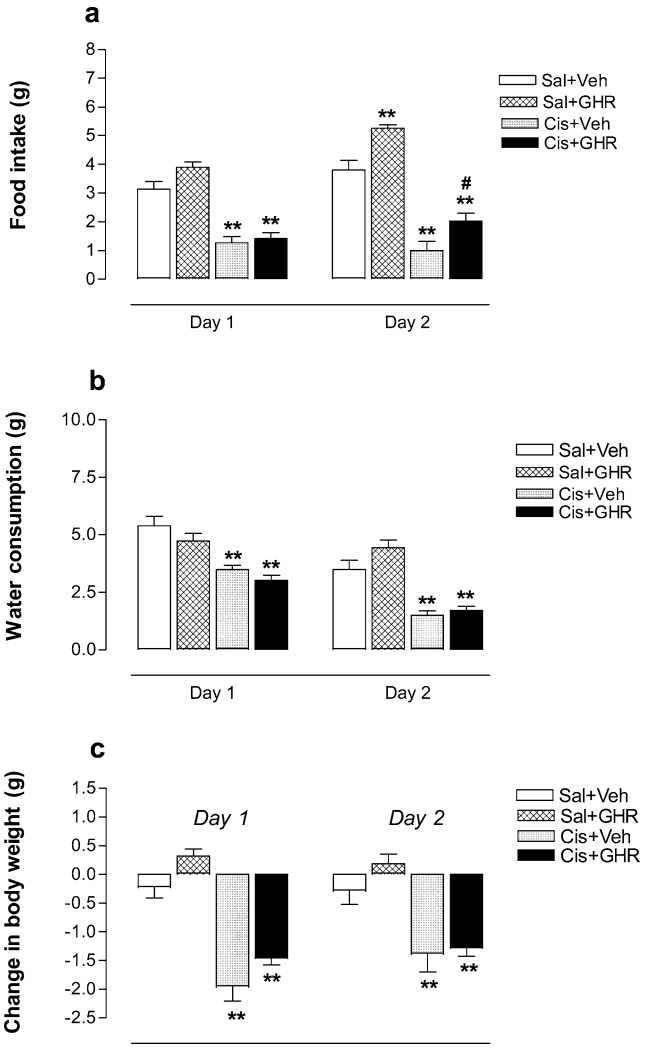

Fig. 3 Effect of 2-day ghrelin treatment (1 mg/kg, i.p. twice a day) on daily food intake (**a**), water consumption (**b**) and body weight changes (**c**) in mice treated with cisplatin (20 mg/kg, i.p.). Results are mean ± SEM ($n = 7$–10). **$P < 0.01$ vs. saline + vehicle; #$P < 0.05$ vs. cisplatin + vehicle; One-way ANOVA followed by Dunnett's test

Effects of 2-day ghrelin treatment on gastric emptying in mice

Gastric contents were collected 48 h post cisplatin injection. The wet weights of gastric content in cisplatin-treated mice were significantly higher than that for the saline-treated mice (saline: 0.06 ± 0.01 g; cisplatin: 0.46 ± 0.04 g, $P < 0.01$). Calculation of the GEI revealed a significant, 53% decrease in GEI in the cisplatin-treated animals as compared to the saline-treated mice ($P < 0.01$, Fig. 4). Ghrelin administration (1 mg/kg, twice daily for 2 days) to cisplatin-treated mice increased the GEI by 31% ($P < 0.01$, compared to cisplatin-treated mice which did not receive ghrelin). There were no statistically significant differences in GEI between cisplatin/ghrelin-treated and saline/vehicle- or saline/ghrelin-treated animals (Fig. 4).

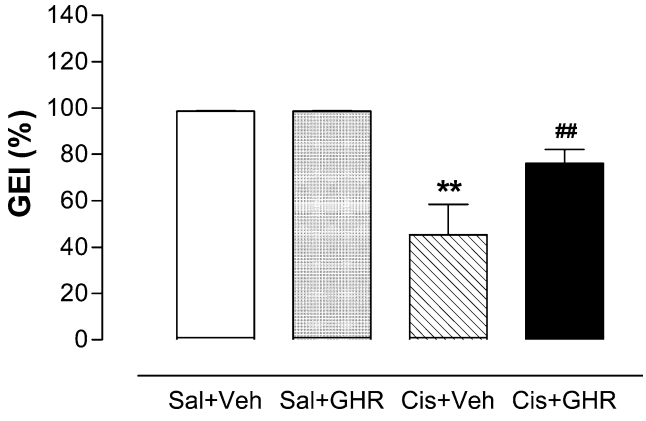

Fig. 4 Effect of ghrelin (1 mg/kg twice daily, i.p.) on gastric emptying 2 days post cisplatin administration (20 mg/kg, i.p.) in mice. Results are mean ± SEM ($n = 7$–10). $^{**}P < 0.01$ vs. saline + vehicle; $^{\#\#}P < 0.01$ vs. cisplatin + vehicle (Dunnett's test)

Discussion

Cancer and cancer chemotherapy-related upper gastrointestinal symptoms have been associated with delayed gastric emptying and may include early satiety, anorexia, nausea and vomiting, often referred to collectively as the CADS [36, 37]. In animals, most studies have focussed on the development of models, which isolate only one of these symptoms. In particular, great attention has been paid to the symptom of vomiting, which has been successfully modelled using species such as the ferret or the house musk shrew, *Suncus murinus* [2, 19]. However, the more commonly-used laboratory animals, rats and mice, are incapable of vomiting. Instead, these species display a range of different behaviours in response to emetogenic substances, including taste aversion, appetite suppression and gastric stasis. These symptoms also occur in animals and humans capable of emesis [42].

In our experiments with both rats and mice, we found that with exception of emesis, cisplatin produced a combination of effects broadly similar to those experienced by patients receiving chemotherapy. In particular, anorexia, reduced body weight, activity and delayed gastric emptying were all observed. The reduction in feeding induced by cisplatin in both rats and mice is consistent with that reported by others in these species. In rats, our studies also suggest that the reduction in feeding behaviour occurs concomitantly with a reduced locomotor behaviour, which may represent a further index of malaise. Finally, our assessment of the rate of gastric emptying in mice, suggests that cisplatin also induces marked gastric stasis that may be an index of the emesis experienced by animal species incapable of vomiting e.g., [24, 29, 33, 38]. Interestingly, species capable of emesis (e.g., humans) also experience gastric stasis during the sensation of nausea and gastric stasis can, in turn cause nausea and vomiting [27] which itself reduces food intake. Overall cisplatin induces a range of effects in rodents with similarities to CADS.

In both rats and mice, our experiments show that intraperitoneal injection of ghrelin can induce a small increase in feeding in animals not treated with cisplatin, as observed during the light-phase when normal feeding behaviour tends to be reduced in rodents, relative to their night-time feeding habits. Further, after suppression of feeding by cisplatin, this orexigenic effect of ghrelin is markedly enhanced. An ability of ghrelin to increase food intake, including in normally fed animals, has been previously demonstrated in both rats and mice by several other investigators [13, 44, 50], but in addition, our observations lend support to those of Neary et al. [35], who reported an ability of ghrelin to increase energy intake and meal appreciation in cancer patients receiving a buffet meal.

In rodents, the mechanisms by, which systemically administered ghrelin can increase feeding behaviours are not fully understood. Considerable attention has been paid to the ability of ghrelin to increase feeding after application to the arcuate nucleus of the hypothalamus, where neurones expressing the orexigenic neuropeptides neuropeptide Y (NPY) and agouti-related protein (Agrp) respond to ghrelin by increasing their firing rate [9, 41, 51]. However, appetite regulatory signals from the gut are also conveyed to the hypothalamus via the vagal afferent neurones to the nucleus of the solitary tract in the brainstem [52]. Further, vagotomy has been shown to eliminate the stimulatory effects on feeding induced by systemically administered ghrelin [3] and the mouse form of ghrelin has been shown to have only a poor ability to cross the mouse blood-brain barrier and enter the brain [5]. Together, these observations may suggest that in our experiments with systemically administered ghrelin, the effects observed are likely to be mediated via the vagus nerve, known to express receptors for ghrelin, at least on those fibres projecting from the stomach [45]. Date et al. [12] have shown co-localisation of the growth hormone secretogogue receptor and the cholecystokinin A receptor on vagal afferents and peripheral interactions between ghrelin and CCK in the regulation of food intake in the rat in response to intravenous ghrelin or CCK administration. In addition, a role for the vagus nerve in mediating the effects of ghrelin is also consistent with an ability of ghrelin to increase feeding when injected into the dorsal vagal complex [16]. However, Ruter et al. [44] showed that systemically administered (i.p.) ghrelin increased feeding in rats and increased c-fos-like immunoreactivity in the arcuate and paraventricular nuclei of the hypothalamus, but not in the nucleus tractus solitarius or in the area postrema, where abdominal vagal afferent neurones predominantly project [17] and a similar result was obtained by Wang et al. [50] using peripherally administered (i.p.) ghrelin in the mouse at a dose comparable to that in the present study. In contrast, Chen et al. [8] reported that the effects of peripherally (i.p.) administered acylated ghrelin on food intake in the rat were blocked by perivagal capsaicin treatment and were associated with an increase in c-fos expression in the nucleus tractus solitarius. It is not yet

possible to reconcile these apparently conflicting observations but clearly both the hypothalamus and the vagus remain the most likely sites for mediating the effects of the peripherally administered ghrelin used in the present study.

In our experiments with rats and mice treated with cisplatin, it is not clear to what degree the increase in food intake induced by ghrelin is the result of a direct orexigenic effect which would be experienced by animals irrespective of their ability or inability to vomit, or to an indirect effect caused by a reduction in the sensation of nausea but observed in rodents only as a change in feeding behaviour and gastric stasis. However, we observed an ability of ghrelin to restore rat locomotor activity suppressed by treatment with cisplatin, to a level, which was not significantly different from the control animals. These observations, together with the increase in feeding behaviour, suggest a generalised improvement in the level of malaise experienced by the rats.

In the mouse model of chemotherapy-associated dyspepsia, ghrelin was found to significantly increase the rate of gastric emptying. These observations are consistent with repeated demonstrations, by others, of a gastric prokinetic activity of ghrelin in both normal animals and in rats with gastric stasis (see Introduction for references). In our experients, the cisplatin-treated mice developed gastric stasis and anorexia, so it was inappropriate to measure the rate of gastric emptying by withdrawing food for a set time period and then re-feeding to assess the gastric emptying rate. Consequently, we measured the amount of food consumed within the last 24 h prior to the administration of ghrelin, to calculate an emptying rate referred to as the GEI. In addition to increasing the GEI, ghrelin tended to reduce the weight loss experienced by the cisplatin-treated mice, although the latter observations did not reach statistical significance. This may be due to the short duration of the treatment (2 days). It is important to note that in the present study, ghrelin increased daily food intake and increased gastric emptying in mice over a 2-day treatment period. Although from our experiments it is not possible to make comment on the degree of exposure of the ghrelin receptors to the ghrelin administered, our observations would suggest that the response to ghrelin is not a short-lived event. Such an observation contrasts with the rapid down-regulation of the receptor observed during the presence of ghrelin in cultured pituitary cell cultures [30] and with the desensitisation of the recombinant ghrelin receptor transfected into a host cell e.g., [7, 39]. The explanation for such an apparent mismatch is not understood, but one possibility is that rates of receptor desensitisation are somehow exaggerated when measurements are made in vitro, especially when using recombinant cell systems. This possibility is suggested by the successful demonstration of a prolonged human gastric prokinetic activity in response to a derivative of erythromycin acting at the motilin receptor [49], which when assessed in vitro, was shown to markedly desensitise at the receptor [28]. The close structural relationships between motilin and ghrelin receptors, as well as our present data, suggests that prolonged biological activity to ghrelin will also occur in vivo.

In summary, the results from the present study suggest that it is possible to model certain attributes of the cancer chemotherapy-induced dyspepsia syndrome in rats and mice. Using peripherally administered, relatively high doses of the gastric hormone ghrelin, we were able to demonstrate that the reductions in feeding, activity, gastric emptying and weight loss induced by cisplatin could be reduced. These observations make the activation of ghrelin receptors a potentially valuable target for the treatment of cancer and cancer chemotherapy-associated dyspepsia syndrome but further studies are required to investigate the effect of lower doses of ghrelin on each of the parameters measured in this study. This potential therapeutic consequences of ghrelin receptor activation is supported by the observation that cisplatin administration in the rat using an identical protocol to that used here induces up-regulation of the ghrelin receptor in both the hypothalamus and stomach within 2 days of administration [34] and the recent finding in the ferret that i.c.v. ghrelin can transiently reduced cisplatin—induced emesis [43].

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