original article

Diabetes, Obesity and Metabolism 2012.

ORIGINAL ARTICLE

Liraglutide: short-lived effect on gastric emptying—long lasting effects on body weight

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Aim: Previous studies with the novel once daily glucagon-like peptide-1 (GLP-1) analogue liraglutide and the GLP-1 receptor agonist exenatide have revealed profound insulinotrophic and antidiabetic effects, but also potent effects on gastric emptying (GE) and long-term and lasting reductions in body weight. In this study, we examined the acute and chronic effects of two different GLP-1 analogues with different pharmacokinetic profiles on GE, food intake and body weight.

Methods: On the basis of a series of dose-finding studies, the doses of exenatide and liraglutide with similar acute anorectic effects were identified. GE was assessed using a standard acetaminophen release assay. After the acute test, rats were dosed bi-daily for 14 days in which period food intake and body weight was monitored. On day 14, the GE rate was reassessed.

Results: While both compounds exerted robust acute reductions in GE, the effect was markedly diminished following 14 days of dosing with liraglutide. In contrast, exenatide-treated rats still displayed a profound reduction in GE at the 14-day time-point. Both compounds exerted similar effects on body weight.

Conclusion: The data suggest that the 'gastric inhibitory' GLP-1 receptors in rats are subject to desensitization/tachyphylaxis but that this effect is dependent on full 24-h exposure as obtained by liraglutide. The body weight-lowering effects of GLP-1 receptor stimulation are not subject to desensitization. These data indicate that regulation of appetite signals in the brain, and not GE, is the main mechanism for liraglutide-induced weight loss.

Keywords: antidiabetic drug, appetite control, exenatide, GLP-1, GLP-1 analogue, incretin therapy

Date submitted 30 August 2011; date of first decision 5 October 2011; date of final acceptance 28 December 2011

Introduction

Glucagon-like peptide-1 (GLP-1) analogues are emerging as an important drug class for the treatment of diabetes and potentially also obesity [1]. Blood borne GLP-1 is derived from endocrine L-cells lining the intestines and secreted in response to a meal [2]. GLP-1 not only acts as a powerful incretin (stimulating glucose induced insulin secretion) but GLP-1 also inhibits glucagon secretin from pancreatic α cells and gastric emptying (GE) (for review see Refs [3–4]). Endogenous GLP-1 has also recently been shown to play a role as a satiety factor [5] in agreement with a wealth of animal and human studies showing food intake lowering effects of peripherally administered GLP-1 (for review see Refs [3,6]). Clinical use of native GLP-1 is hampered by its rapid inactivation by dipeptidylpeptidase IV resulting in a half-life of a few minutes in man [7]. Recently, however, the once daily GLP-1 analogue liraglutide and the twice daily GLP-1 receptor agonist exenatide were approved for the treatment of type 2 diabetes mellitus. Furthermore, a number of studies have shown clinically relevant weight loss following treatment with liraglutide or exenatide in both diabetic [8] and obese patients [9] in agreement with the first report of GLP-1-induced satiety and lowered energy intake [10].

The underlying mechanisms that mediate the weight loss elicited by liraglutide and exenatide are not entirely clear, but it is a general belief that this could be a combination of effects on the gastrointestinal tract and central GLP-1 receptor expressing neurons [3,9,11]. It has previously been shown that the most important physiological mechanism of GLP-1-induced lowering of postprandial glycaemia is inhibition of GE, which leads to a delayed entry of glucose into the circulation [12,13] and that blocking the GLP-1-induced inhibition of GE lowers its incretin effect markedly [14]. This has also led to speculations that the body weight and appetite-reducing effects of liraglutide and exenatide might partly be related to the ability of GLP-1 to decrease GE and reduce gastric motility [12].

Studies with liraglutide and exenatide in mini-pigs [15] and rhesus macaques [16] have showed potent effects of these analogues on particularly meal size, which could be indicative of gastric inhibition. In clinical studies, the major side effect of both exenatide and liraglutide is mild nausea, which seems to occur in a dose-dependent fashion and be transient in nature [8,9,17]. Although it is tempting to speculate that the cause of nausea observed in clinical trials is a consequence of the marked gastric inhibitory effects of GLP-1 and GLP-1 analogues

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this actually remains to be elucidated. Clinical data suggest that the pharmacodynamics of nausea is highly dependent on the pharmacokinetic profile of the specific GLP-1 agonists used. For instance, the combination of the once daily liraglutide with a slow up-titration seems to facilitate high tolerance levels evidenced by a markedly lower incidence of patients reporting nausea [9]. Furthermore, it has been reported that the incidence of nausea is reduced when patients are shifted from exenatide to liraglutide [18] emphasizing the potential importance of different exposure profiles on the incidence of reported nausea.

The transient nature of nausea observed in clinical trials with liraglutide and the possibility that nausea and inhibition of GE are causally related prompted us to investigate the effects of liraglutide on GE following acute and chronic administration. Furthermore, it is debated whether the effect of GLP-1 analogues on weight loss is caused by inhibition of gastric motility [12,19] or because of interaction with specific neurocircuitries involved in the regulation of feeding behaviour. Because the reported incidence of nausea is transient in patients treated with liraglutide [8–9], we speculated that liraglutide-induced inhibition of GE is transient as well. This study was initiated to elucidate potential drug-related differences on GE following acute and chronic treatment with equal-efficacious (with respect to body weight) bi-daily doses of liraglutide and exenatide.

Materials and Methods

Experimental Animals

All animal experiments were conducted in accordance with internationally accepted principles for the care and use of laboratory animals, and in compliance with personal animal licenses for Jacob Jelsing (2008/561-1565) issued by the Danish Committee for Animal Research. A total of 32 male Sprague Dawley rats (10-weeks old) were used for the acute dose-finding study and additional 32 male Sprague Dawley rats (10-weeks old) were used for the 14 days chronic study. Subsequently, 30 rats were included to determine the optimal time-point for GE profiling (the time where inhibition of GE was most pronounced following subcutaneous administration of liraglutide) to exclude potential differences in pharmacokinetics. All animals were obtained from Charles River (Charles River Laboratories, Sulzfeld, Germany). Upon arrival at the animal unit, the rats were housed two per cage under a normal light cycle (light from 06:00 to 18:00 hours) at controlled temperature conditions for 1 week. Following acclimatization, the animals were transferred and housed individually in MaNi Feedwin cages [20]. A minimum of 7 days habituation to the system was allowed before the experimental procedures. The animal room environment was controlled (targeted ranges: temperature $22 \pm 2\ ^{\circ}C$; relative humidity $50 \pm 10\%$; light/dark cycle: 12-h light, 12-h dark, lights on from 07:00 to 19:00 hours. Rats had *ad libitum* access to Altromin powdered diet (Brogaarden, Lynge, Denmark) and tap water.

Acute Effects on Food Intake—Dose-Finding

One day before the experiment, rats were randomized according to body weight into seven experimental groups: Group 1 (Vehicle), Group 2 (liraglutide, 30 μg/kg), Group 3 (liraglutide, 100 μg/kg), Group 4 (liraglutide, 300 μg/kg), Group 5 (exenatide, 1 μg/kg), Group 6 (exenatide, 3 μg/kg) and Group 7 (exenatide, 10 μg/kg). Compounds were obtained from Bachem (Exendin-4, H87-30, Lot. 1013774, Bubendorf, Switzerland) and Novo Nordisk (Liraglutide, Lot. TQ50561, Maaloev, Denmark) and were diluted in phosphate-buffered saline added 0.5% bovine serum albumin.

In the evening on the experimental day (immediately before lights out), rats were randomized based on body weight and dosed with liraglutide, exenatide or vehicle in a randomized fashion [intravenous tail injection (2 ml/kg)] using a 27 g × 5/8″ needle (Monoject™, Kendall, Mansfield, Massachusetts, USA) connected to a 1-ml syringe (luer-lock™, Becton, Mansfield, Massachusetts, USA). Food (g) and water intake (lickometers) and locomotor activity (recorded as consecutive beam brakes) was recorded online every fifth minute for 18 h using an online computerized feeding system using digital weighing cells and lickometers. For cumulative food intake, the mean data for 30 min (six consecutive measurements) were extracted for the next 18 h. Following a 5 days drug wash-out period, all animals were re-randomized based on body weight before a second and third experiment was performed testing the same doses providing a total number of 8–13 individual datasets per group. Finally, animals were killed by decapitation under CO_2/O_2 anaesthesia.

Gastric Emptying and Chronic Effects on Body Weight

One day before the experiment rats were randomized according to body weight into four experimental groups (n = 8 per group): Group 1 (Vehicle—one i.v. injection followed by s.c. injections bi-daily), Group 2 (liraglutide, 200 μg/kg—one i.v. injection followed by bi-daily s.c. injections), Group 3 (exenatide, 3 μg/kg—one i.v. injection followed by bi-daily s.c. injections) and Group 4 (exenatide, 10 μg/kg—one i.v. injection followed by bi-daily s.c. injections). The acute GE experiment was performed in the morning with food being removed 14 h before dosing (i.e. at 22:00 hours the night before). Rats were injected intravenously (via the tail vein, 2 ml/kg) 30 min before an oral gavage with acetaminophen (40 mg/kg; 10 ml/kg). Blood samples (100 μl) were obtained from the tail vein at 15, 30, 45, 60, 90, 120 and 180 min after administration of acetaminophen. Following the last blood sample food was reintroduced and all animals continued into a 14-day bi-daily dosing study with food intake, water intake and locomotor activity being continuously monitored throughout the study period in the MANI-Feedwin system. Body weight was recorded daily in the morning. In the evening at day 13, food was removed from all rats. The next morning, animals were dosed subcutaneously (2 ml/kg) with vehicle, liraglutide (200 μg/kg) and exenatide (3 μg/kg or 10 μg/kg) and 30 min later gavaged with acetaminophen for a chronic GE profiling using the same procedures as described above. Finally, animals were killed by decapitation under CO_2/O_2 anaesthesia.

Validation Studies

To test whether potential differences in chronic GE following the final (day 14) subcutaneous administration of liraglutide

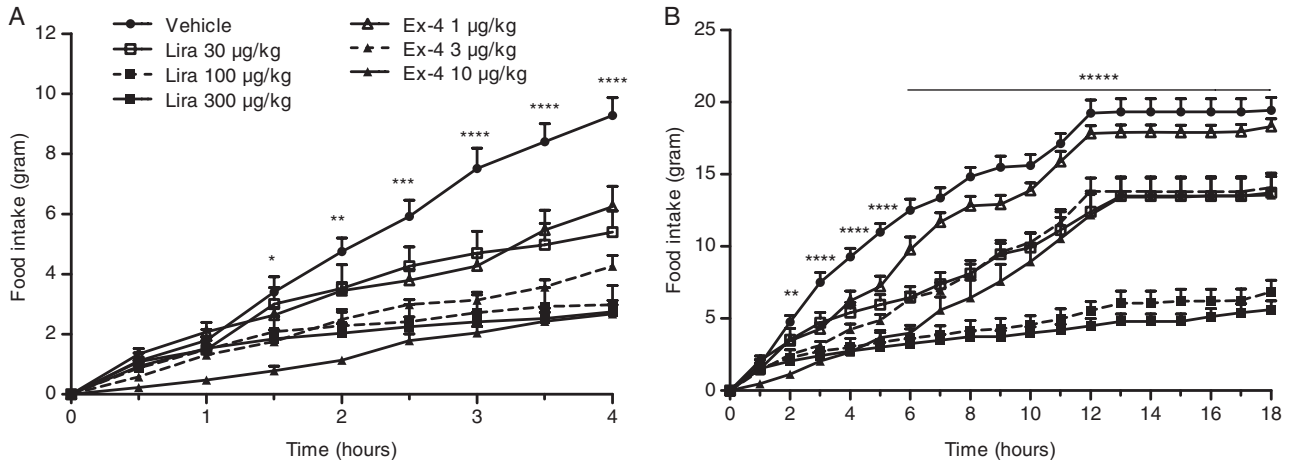

Figure 1. Acute food intake following intravenous administration of liraglutide (30, 100 or 300 µg/kg) or exenatide (1, 3 or 10 µg/kg) immediately before lights out ($t = 0$). (A) Food intake during the first 4 h following compound administration. (B) Food intake during the first 18 h following compound administration. Statistical analyses (two-way ANOVA), $p < 0.05$, *exenatide 10 µg/kg and liraglutide 300 µg/kg, **exenatide 3,10 µg/kg and liraglutide 100,300 µg/kg, ***exenatide 1,3,10 µg/kg and liraglutide 100,300 µg/kg, ****exenatide 1,3,10 µg/kg and liraglutide 30,100,300 µg/kg significantly different from vehicle, *****exenatide 3,10 µg/kg and liraglutide 30,100,300 µg/kg significantly different from vehicle.

and exenatide could be due to the different pharmacokinetic profile of the two compounds, a follow-up study was pursued to profile GE 30, 60, 120 and 240 min (n = 6 per group) following a subcutaneous administration of liraglutide (200 µg/kg). Optimally, this study could have been performed in a pilot study, or alternatively by using the i.v. route of administration at both time-points in the original set-up (which, however, does not reflect the clinically relevant route of administration). Subsequently, 12 animals (n = 6 per group; vehicle bi-daily, liraglutide 200 µg/kg bi-daily) continued in a chronic 14-days dosing design before a final GE was performed at the optimal time-point (liraglutide administered 120 min before acetaminophen administration).

Acetaminophen Assay

Plasma levels of acetaminophen were determined in duplicates using a commercially available acetaminophen Kit (MULTIGENT, B2K996, Abbot Laboratories, Abbott Park, IL 60064, USA),

Statistical Analysis

In vivo data were analysed using the MaNi FeedWin database software. *In vivo* data and acetaminophen data were subsequently subjected to relevant statistical analyses using StatView or GraphPad software. Results are presented as mean ± s.e.m. (standard error of the mean). Statistical evaluation of the data was carried out using one-way or two-way analysis of variance (ANOVA) with appropriate *post hoc* analysis between control and treatment groups in cases where statistical significance was established (level of significance, $p = 0.05$). Statistical results for food intake and body weight are given for every 30 min (0–18 h data) or daily (1–14 day study). Statistical data for acetaminophen release is provided for every time-point.

Results

The Acute Effect on Food Intake

Intravenous administration of liraglutide (30, 100 or 300 µg/kg) and exenatide (1, 3 or 10 µg/kg) reduced food intake in a dose-dependent manner (figure 1). The effect of exenatide on food intake was most pronounced within the first four hours post-dosing (figure 1A) and only 3 and 10 µg/kg significantly reduced food intake at the end of the observation period (18 h after dosing; figure 1B). In contrast, all doses of liraglutide exerted marked reductions in food intake that lasted for the entire observation period consistent with the longer half-life of this GLP-1 analogue (figure 1).

Gastric Emptying Profiling and the Chronic Effect on Body Weight

For liraglutide, a dose of 200 µg/kg was used as this has been shown to be effective for chronic food intake and body weight regulation in rats [15,21]. On the basis of the acute food intake data 3 µg/kg and 10 µg/kg doses of exenatide were chosen as they both led to a marked reduction in food intake comparable to the liraglutide 100–300 µg/kg. Moreover, the ratio between liraglutide and exenatide is comparable to the clinically dosing regime in diabetes (1.8 mg vs. 2 × 10 µg daily).

Both exenatide (3 and 10 µg/kg i.v.) and liraglutide (200 µg/kg i.v.) potently inhibited GE rate, as shown by the ability to reduce plasma acetaminophen levels over time (figure 2A). This is also clear when expressed as area under the acetaminophen plasma level curve (a measure of total inhibition during the 3-h period, figure 2B) showing an almost 90% inhibition for liraglutide, with slightly less and dose-dependent effects of exenatide.

During the following 14-days subcutaneous dosing both exenatide- and liraglutide-treated rats lost weight in comparison to vehicle (figure 3A). In agreement with the acute food

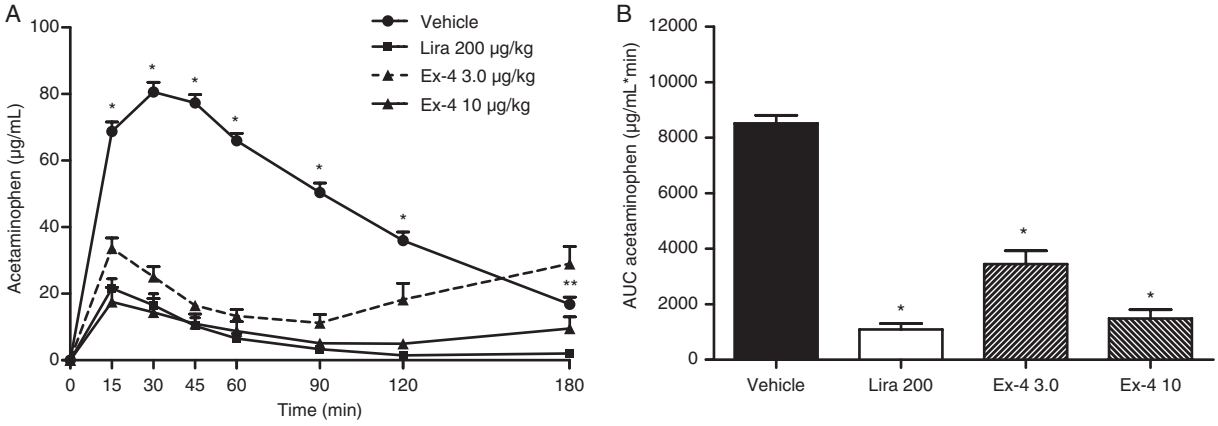

Figure 2. (A) Plasma levels of acetaminophen following intravenous administration (at $t = -30$ min) of vehicle, liraglutide 200 µg/kg or exenatide 3 or 10 µg/kg. Acetaminophen were given perorally at $t = 0$. (B) Area under the curve (0–180 min). Statistical analyses, Fig 2A (two-way ANOVA), Fig 2B (one-way ANOVA), $p < 0.05$, *exenatide 3,10 µg/kg and liraglutide 200 µg/kg, **exenatide 10 µg/kg and liraglutide 200 µg/kg significantly different from vehicle.

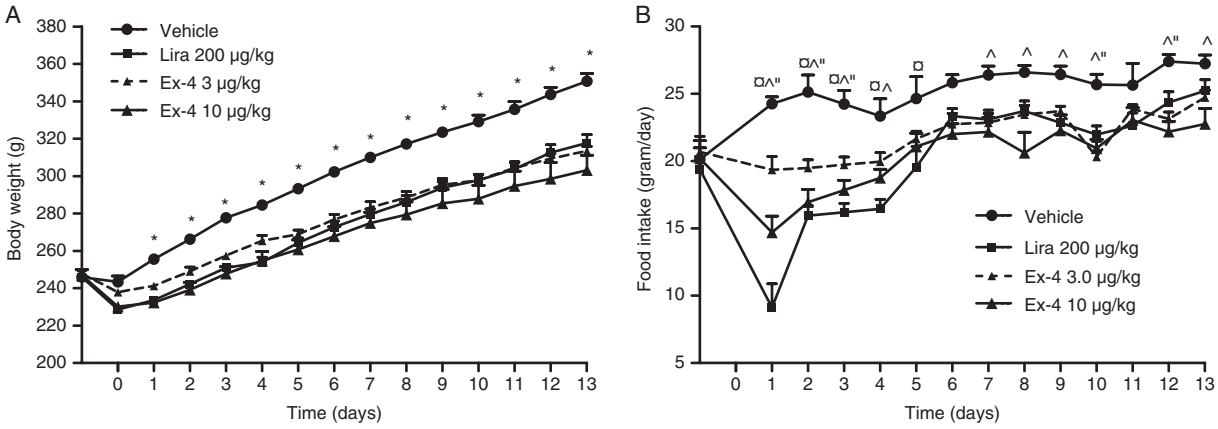

Figure 3. (A) Body weight and (B) daily food intake during the 14-day dosing study. Statistical analyses (two-way ANOVA), $p < 0.05$, * all groups; ¤ liragutide 200 µg/kg, ''exenatide 3 µg/kg and ^exenatide 10 µg/kg significantly different from vehicle.

intake data, liraglutide had the strongest effect on food intake initially with food intake being nearly similar across compound-treated groups following the first approximately 48-h food (figure 3B). The effect of liraglutide on food intake did not reach significance throghout the experiment but tended to be reduced at all days. Similarly, the effect of 3 µg/kg exenatide only reached significance at days 1–3, 10 and 12. However, at the end of the treatment period body weight was roughly similar between all drug-treated groups and approximately 10% less than vehicle dosed rats (figure 3A).

Detailed meal structure analyses did reveal slight differences between compounds with exanatide leading to acute reductions in both meal size and meal duration, as well as on meal size compared to vehicle (Table 1).

The study was concluded with a final chronic GE profiling 30 min following the final s.c. administration of compound. The s.c. administration was selected to mimic the clinical situation as closely as possible. In contrast to the acute GE profiling, only a slight inhibition of approximately 5% was observed for the liraglutide-treated rats 30 min following administration. For exenatide, the GE was still dramatically reduced (figure 4A, B) and comparable to that seen after acute administration (compare figure 4A, B to figure 2A, B)

Validation Study at Time of Maximal Exposure

The markedly reduced effect of liraglutide on GE at the final time-point prompted us to ascertain that the observed effects of liraglutide and exenatide on GE were not a consequence of differences in pharmacokinetics. Hence the optimal time (i.e. the time-point were the inhibitory effect of liraglutide on GE are most pronounced) was determined in a new set of animals. These data clearly showed that the effect of subcutaneously administered liraglutide is most pronounced when administered 120 min before the acetaminophen profiling (figure 5A) with a significant inhibition of acetaminophen release at all time-points during the test period. This indicate that the observed lack of effect of liraglutide in the chronic study at $t = 30$ min could be partly attributable to lack of acute exposure (although the long half-life of liraglutide and the bi-daily dosing regimen leads

Table 1. Meal structure analyses day 1, 6 and 12.

	Day 1				Day 6				Day 12			
	Vehicle	Liraglutide	Exenatide3	Exenatide10	Vehicle	Liraglutide	Exenatide3	Exenatide10	Vehicle	Liraglutide	Exenatide3	Exenatide10
Food intake (g)	24.3 ± 0.50	9.12 ± 1.76*	19.35 ± 1.00*	14.70 ± 1.22*	25.84 ± 0.58	23.35 ± 0.55	22.74 ± 0.82	22.02 ± 0.77*	27.4 ± 0.53	24.36 ± 0.78	23.13 ± 0.53*	22.18 ± 0.78*
Meal size (g)	5.54 ± 0.46	3.29 ± 0.21*	2.89 ± 0.42*	2.51 ± 0.24*	2.49 ± 0.14	2.23 ± 0.14	1.79 ± 0.13*	1.69 ± 0.15*	2.32 ± 0.21	1.93 ± 0.12	2.03 ± 0.22	1.68 ± 0.082*
Meal duration (min)	48.26 ± 4.92	37.88 ± 2.66	26.29 ± 2.95*	24.71 ± 2.68*	22.63 ± 1.90	25.49 ± 1.81	18.88 ± 1.55	17.19 ± 0.91	19.86 ± 2.47	19.10 ± 1.59	18.89 ± 2.06	16.81 ± 1.04
Intermeal interval (min)	189.9 ± 36.0	135.0 ± 13.24	107.7 ± 14.36	141.9 ± 23.86	96.29 ± 8.14	99.41 ± 4.78	78.91 ± 4.32	79.77 ± 7.32	96.28 ± 7.38	79.19 ± 6.04	85.60 ± 9.34	85.63 ± 5.45

Data collected for 23 h from 09:00 to 08:00 hours; light/day cycle 12/12, lights off at 19:00 hours (mean values ± s.e.m).
*Statistical significant different from vehicle.

to full 24-h exposure). Consequently, a new chronic dosing study was performed with 14 days of s.c. dosing with liraglutide followed by a terminal GE profiling. The data corroborated the previously obtained findings, showing a complete reduction in the effect of liraglutide on GE following chronic exposure (figure 5B).

Discussion

This study shows that the GLP-1 receptor agonists exenatide and liraglutide exerts potent anorectic and gastric inhibitory effects and that doses with equivalent anorectic efficacy cause comparable inhibition of GE in the acute setting. However, while inhibition of GE following drug administration persisted in rats treated for 14 days with exenatide, it was markedly diminished in liraglutide-treated rats suggesting that desensitization/tachyphylaxis of gastric inhibition depends on a full 24-h receptor exposure. The fact that exenatide and liraglutide caused a decrease in food intake and similar effects on weight loss, despite the very different effects on GE, indicate that inhibition of GE do not play a major role in neither the acute or chronic effects on body weight exerted by these compounds.

The findings that chronic receptor exposure leads to desensitization of the gastric inhibitory effects is in line with a recent study performed in humans showing that the gastric inhibitory potential of GLP-1 was markedly decreased following a relatively short 8-h infusion [22]. Nauck et al. [22] proposed that because of the short timeframe the gastric inhibitory effects of GLP-1 were subject to tachyphylaxis rather than to desensitization which usually depends on receptor internalization and degradation. In the current study the effect of liraglutide and exenatide on GE were assessed only at study start and at termination 14 days later. Hence, we are not able to determine exactly when the effect wears off. However, as the effects of liraglutide on GE were markedly diminished following 14 days of dosing in the current study and also diminished in the acute study by Nauck et al. [22], it is possible that both mechanisms of tachyphylaxis and receptor desensitization are involved in the process that leads to the marked reduction of the response in rats subjected to full 24-h GLP-1 receptor exposure. Interestingly, the gastric inhibitory effects of exenatide actually appeared to be exaggerated at the 14-day timepoint when compared with the initial GE indicating that intermittent receptor stimulation could lead to an opposite sensitizing effect.

As acute administration of both liraglutide and exenatide leads to profound reductions in food intake in rats [23–25] it is conceivable that some of the acute effects are mediated by their inhibitory effects on GE. However, GE is not responsible for the long-term reductions in body weight obtained by liraglutide. If weight loss was driven by inhibition of GE animals treated with liraglutide and exenatide would display regaining weight and increasing food intake at the end (as a normal counter-regulatory effect following a 10% body weight loss). All compound-treated groups showed a very similar body weight at day 14, despite very different GE profiles, providing further support for the notion that the effects on GE can be separated from the effects on body weight. As the gastric

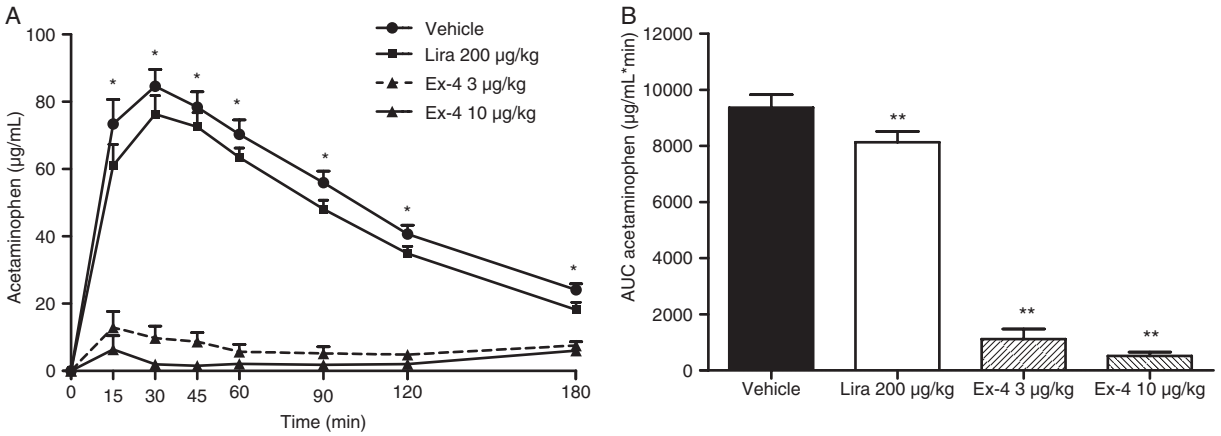

Figure 4. (A) Plasma levels of acetaminophen following subcutaneous administration (at $t = -30$ min) of vehicle, liraglutide 200 µg/kg or exenatide 3 or 10 µg/kg on day 14 in chronically administered animals. Acetaminophen were given perorally at $t = 0$. (B) Area under the curve (0–180 min). Statistical analyses, Fig 4A (two-way ANOVA), Fig 4B (one-way ANOVA), $p < 0.05$, *exenatide 3,10 µg/kg significantly different from vehicle, **exenatide 3, 10 µg/kg and liraglutide 200 µg/kg significantly different from vehicle.

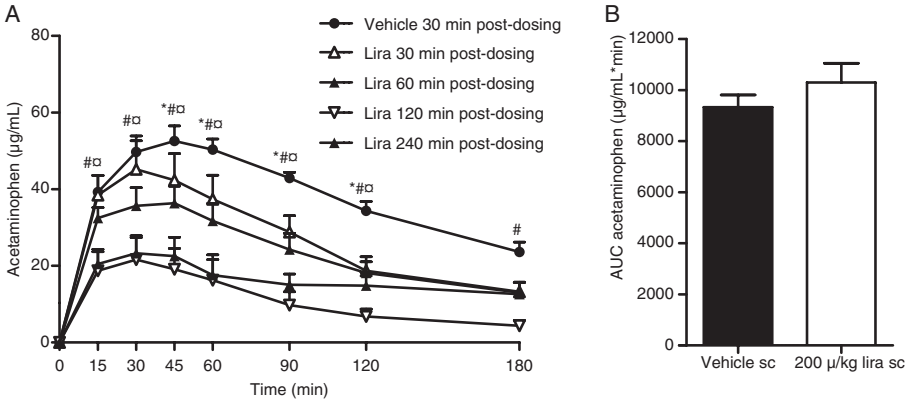

Figure 5. Testing the time-point where the inhibitory effect of liraglutide on GE is most pronounced. GE profiling of acetaminophen tests performed 30, 60, 120 and 240 minutes following administration with liraglutide. (A). The effect of liraglutide on GE (administered subcutaneously 120 min before GE test) following 14-days exposure (B). Statistical analyses, one-way ANOVA, $p < 0.05$, *Lira60 min, #Lira120 min and ¤Lira240 min post dosing significantly different from vehicle.

inhibitory effects and the body weight-lowering effects of GLP-1 analogues can be dissociated, these effects must be mediated by different receptor populations.

GLP-1 receptors are expressed on the vagal afferents [26] and desensitization of these receptors may be responsible for the subsiding gastric inhibitory effects [22]. However, while it has been shown that the efferent vagus is necessary for GLP-1 elicited gastric inhibitory effects [27,28] it was recently showed that the inhibitory effects of GLP-1 on gastric motility in the pig persists after vagal deafferentiation [29] suggesting that other centrally located GLP-1 receptors are involved. This is supported by recent data showing that GLP-1 can directly activate vagal preganglionic motorneurons in the brainstem leading to gastric inhibitory effects [30].

The GLP-1 receptors mediating the appetite inhibitory effects of GLP-1 are also a matter of discussion. It has been suggested that vagal GLP-1 receptors are involved in mediating the satiating effects of peripherally administered GLP-1 [31], but studies with infusions of small doses of GLP-1 into either the portal vein or the superior vena cava in vagal deafferentiated rats has suggested that central rather than peripheral GLP-1 receptors are involved [32]. In line with the latter view peripheral administration of GLP-1 [33] and exendin-4 [34,35] leads to c-Fos activation in a number of brainstem sites that express GLP-1 receptors [36] being accessible to peripherally administered GLP-1 [37]. Moreover, hypothalamic GLP-1 receptors could also play a role with GLP-1 receptors on cocaine and amphetamine regulated transcript of proopiomelanocortin (CART/POMC) neurons in the arcuate nucleus known to respond to other peripherally circulating hormones, for example, leptin [38].

From a clinical perspective, it is very interesting that chronic exposure of the GLP-1 receptor abolish the gastric inhibitory effects. Clinical trials report that the most frequent adverse effects of GLP-1 agonists are gastrointestinal intolerance, especially nausea [8,9]. However, this intolerance subsides soon in most patients. Also in this study, signs of visceral illness were only noted for the first 1–2 days (data not shown).

Thus, nausea cannot explain the weight loss effect of GLP-1 analogues. Interestingly, whereas liraglutide once daily and exenatide twice daily produce similar initial rates of nausea, there is a significantly greater rate of nausea in the exenatide-treated patients following a half year of treatment [8]. It is tempting to speculate that a continued marked effect on GE as seen with exenatide is causing nausea in some patients. However, at present there exist no conclusive evidence for a direct and causative link between inhibition of GE and nausea [39,40]. In this study, we used paracetamol uptake as a measure of GE. While several human studies also uses this test [41–43], it has to be noted that it only takes into account liquid emptying. Studies that compare liquid emptying using the paracetamol test and solid emptying using scintigraphy acutely and chronically could be warranted to fully understand the relationship if GE is somehow implicated in the aversive effects of GLP-1 analogues.

In this study, we did not measure blood glucose as these animals were not diabetic, and the study was focused on understanding the potential involvement of GE in the body weight-lowering effect of GLP-1 analogues. However, the marked difference in GE between liraglutide and exenatide correlates very nicely to the difference in glucose profiles with the two drugs. Exenatide has a more marked effect on postprandial blood glucose, whereas liraglutide has stronger effect on fasting blood glucose, and also on HbA1c [8].

The differential response in GE we report here is most probably because of differences in pharmacokinetics, and it appears that the desensitization/tachyphylaxis of the gastric inhibitory GLP-1 receptors appears to require a full 24-h exposure. Furthermore, one should bear in mind that to mimic the once daily exposure profile of liraglutide in humans, a twice daily administration is needed in rats because of the shorter half-life of liraglutide in this species [44]. The half-life of exenatide is short and fairly similar between rats and man [45,46].

In conclusion, our data show that a full 24-h receptor exposure is needed to desensitize the gastric inhibitory effects of GLP-1 analogues and that gastric inhibition does not contribute to the weight loss effects of long-acting GLP-1 agonists. We propose that body weight loss elicited by the twice daily GLP-1 receptor agonist exanatide and by the once daily GLP-1 analogue liraglutide is mediated by either brainstem or hypothalamic GLP-1 receptors.

Acknowledgements

Lotte Handgaard Jørgensen and Farida Sahebzadeh at gubra are thanked for their excellent technical assistance.

Conflict of Interest

J. J., N. V., M. T. C. H., K. R. and L. B. K. designed the studies. G. H., M. T. C. H., J. J. and N. V. were involved in the conductance of the *in vivo* studies. G. H. and J.J. are responsible for data analyses. J. J. wrote the first draft of the paper. N. V. and L. B. K. contributed to writing of the paper.

K. R., M. T. C., L. B. K. are full time employees of Novo Nordisk who has developed liraglutide for the treatment of diabetes. All these authors hold minor stock portions, as part of an employee offering programme. J. J. and N. V. are founders and owners of gubra—a contract research organization working with diabetes and obesity research. G. H. is a full time employee at gubra with no ownership. N. V. is a consultant on a scientific committee at Novo Nordisk. J. J., N. V. and G. H. have no stock portions in Novo Nordisk.

Part of the data was previously published as a poster at the American Diabetes meeting 2010, as well as on the European Diabetes meeting 2010.

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