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Glucagon-like peptide-1 receptor agonist administration suppresses both water and saline intake in rats

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Abstract

Glucagon-like peptide-1 (GLP-1) plays an important role in energy homeostasis. Injections of GLP-1 receptor (GLP-1R) agonists suppress food intake, and endogenous GLP-1 is released when nutrients enter the gut. There is also growing evidence that the GLP-1 system is involved in the regulation of body fluid homeostasis. GLP-1R agonists suppress water intake independent of their effects on food intake. It is unknown, however, whether this suppressive effect of GLP-1R agonists extends to saline intake. Accordingly, we tested the effect of the GLP-1R agonists liraglutide (0.05 µg) and exendin-4 (0.05 µg) on water and saline intakes stimulated either by angiotensin II (AngII) or by water deprivation with partial rehydration (WD-PR). Each agonist suppressed AngII-induced water intake; however, only exendin-4 suppressed saline intake. WD-PR-induced water and saline intakes were both attenuated by each agonist. Analysis of drinking microstructure after WD-PR found a reliable effect of the agonists on burst number. Furthermore, exendin-4 conditioned a robust taste avoidance to saccharine; however, there was no similar effect of liraglutide. To evaluate the relevance of the conditioned taste avoidance, we tested whether inducing visceral malaise by injection of lithium chloride (LiCl) suppressed fluid intake. Injection of LiCl did not suppress water or saline intakes. Overall, these results indicate that the fluid intake suppression by GLP-1R activation is not selective to water intake, is a function of post-ingestive feedback, and is not secondary to visceral malaise.

Keywords

Fluid intake; Liraglutide; Exendin-4; thirst

Introduction

Glucagon-like peptide-1 (GLP-1) is produced primarily within the small and large intestines and in the nucleus of the solitary tract (NTS) in the brain (1–5). GLP-1 is an incretin, increasing nutrient-dependent insulin release (6). Because of this effect, GLP-1 receptor (GLP-1R) agonists are used for the treatment of type-2 diabetes mellitus. Although the short half-life of GLP-1 makes it less helpful clinically, more recently developed agonists, such as liraglutide and exendin-4, have longer half-lives and, therefore, better therapeutic potential (7, 8).

The GLP-1 system is also involved in the regulation of food intake. GLP-1R agonists suppress food intake, whereas GLP-1R antagonists induce hyperphagia (9–14). Furthermore, peripheral GLP-1 release is stimulated by nutrients in the gut (15, 16). These findings have

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led to the conclusion that GLP-1 is a feeding-specific satiety signal. This view has been challenged, however, by growing evidence that stimulation of GLP-1R affects other behaviours, including water intake (11, 17–21). Studies from our laboratory extended these findings by showing that the hypodipsic effect of GLP-1R agonists is not secondary to a primary effect on food intake because these drugs suppress overnight water intake even when food is unavailable (21). Nevertheless, additional tests of specificity are required.

To provide additional information about the specificity of the effect of GLP-1R agonists, we used two different methods to stimulate water and saline intakes, central injection of AngII and water deprivation followed by partial rehydration (WD-PR). These experiments tested the hypothesis that administration of GLP-1R agonists suppress water and saline intakes in rats, extending our earlier studies by including different drinking stimuli and by testing for any effect on saline intake. Furthermore, we conducted a series of control experiments to determine if any intake effects could be attributed to the sensation of visceral illness that has been reported to occur after GLP-1R agonist administration (22–24). To the best of our knowledge, this is the first test of GLP-1R agonists on saline intake and the first test for a hypodipsic effect of GLP-1R agonists on WD-PR-stimulated fluid intake.

Methods

Ethical Approval

All experimental protocols were approved by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo and conformed to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Animals

Adult male Sprague-Dawley rats (325–349 g) were purchased from Harlan Laboratories Inc (Indianapolis, IN). Rats were housed in hanging wire mesh cages (Unifab Corporation, Kalamazoo, MI) in a temperature- and humidity-controlled environment. Lights were maintained on a 12:12 light:dark schedule and standard rodent chow and water were available *ad libitum*, except where noted. Each rat was used in only one experiment.

Cannula implantation and placement verification

Rats in Experiments 1, 2, 3, and 5 were implanted with chronic indwelling cannula aimed at the lateral ventricle (LV). Rats were anaesthetised using a combination of ketamine (70 mg/kg, IM; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (5 mg/kg, IM; Spectrum Chemical, Gardena, CA) before being secured in a stereotaxic apparatus. Rats then were given a single injection of carprofen (5 mg/kg, IM; Pfizer Animal Health, New York, NY). A small burr hole was drilled and a guide cannula (26 ga; Plastics One Inc., Roanoke, VA) was implanted using the following coordinates: 0.9 mm posterior to bregma, 1.4 mm lateral to midline, and 1.8 mm ventral to dura. The Cannula was secured to the skull using bone screws and dental cement. Proper cannula placement and patency were verified at least five days post-surgery and after the completion of all test days by measuring the drinking response to an LV injection of AngII (10 ng). Only rats that drank at least 6 ml during these verification procedures were included in the final analyses.

Drug injections and intake measures

Injections were made with a 33 ga injection cannula attached to water-filled PE 50 tubing connected to a 2 µl Hamilton syringe. Injection cannulae were held in place for approximately 30 sec after each injection. Water and saline bottles were weighed immediately before and after testing periods. Total fluid intake was calculated by taking the difference of the pre- and post-bottle weights. Drinking behaviour also was measured

throughout the testing period by recording the number of licks with a contact lickometer (designed and constructed by the Psychology Electronics Shop, University of Pennsylvania, Philadelphia, PA). The lickometer interfaced with a computer using an integrated USB digital I/O device (National Instruments, Inc., Austin, TX) and data were acquired and processed in a MATLAB (MathWorks, Natick, MA) software environment before being ported to excel for final analysis. Bottle spouts were behind an electrically isolated metal plate with a 3.175 mm-wide opening through which the rat needed to lick to reach the spout, minimizing the possibility of non-tongue contact with the spout. Rats were habituated to the modified bottle arrangement for at least 5 d.

Experiment 1: Effect of GLP-1R agonists on AngII-induced water and saline intakes—Rats (n=15) were habituated to two-bottle *ad libitum* access to water and 1.5% saline for at least 5 d prior to testing. Approximately 2 h after lights on, food and fluids were removed and rats received a pretreatment injection of liraglutide (0.05 µg), exendin-4 (0.05 µg), or vehicle (1 µl 0.9% saline) into the LV. These drugs have been shown to have a delayed effect on dark phase water intake (21), therefore, 2 hr after the pretreatment, a test injection of AngII (100 ng) was administered into the LV. Water and saline bottles then were made available, and fluid intake was measured for the subsequent two hours. Food was returned after the two-hour fluid intake test. This experiment was conducted with a repeated measures design. Rats received each treatment condition in a partial Latin squares design and testing days were separated by 3–5 d. Total water intake data from two animals were removed from analysis due to bottle leakage.

Experiment 2: Effect of GLP-1R agonists on modified WD-PR-induced water and saline intakes—Rats were habituated to two-bottle *ad libitum* access to both water and 1.5% saline for at least 5 d prior to testing. The traditional WD-PR procedure (25) was modified as described below for this experiment to account for the longer acting effects of liraglutide and exendin-4 (21). Using a between subjects design, rats had fluids removed 24 hr prior to an injection of liraglutide (0.05 µg; n=5), exendin-4 (0.05 µg; n=5), or vehicle (1 µl 0.9% saline; n=5). Immediately after the injection, rats were given access to 7 ml of water (partial rehydration period). Because our pilot studies found that GLP-1R agonists suppressed water intake during the rehydration period, we modified the WD-PR procedure so that each rat was only allowed to drink 7 ml of water. This volume was consumed by all rats, regardless of treatment condition. After the partial rehydration period, *ad libitum* water and saline, but not food, were returned and fluid intake was measured for two hours. Total water intake data from one animal treated with exendin-4 was removed from analysis due to bottle leakage. Lick patterns also were analysed as described in *Data Analysis* below.

Experiment 3: Does exendin-4 (0.05 µg) or liraglutide (0.05 µg) condition a taste avoidance?—Rats were habituated to a restricted drinking schedule during which they had access to two bottles of water for 90 min each day, beginning approximately 2 h after lights on. After 7 d on this schedule, rats were given access to two bottles of 0.1% saccharine, instead of water, for 90 min. Immediately after saccharine exposure, rats were given an LV injection of liraglutide (0.05 µg; n=7), exendin-4 (0.05 µg; n=6), or vehicle (0.9% saline; n=4), or an ip injection of lithium chloride (LiCl; 0.15 M, 1.33 ml/100 g body weight; n=5). Forty eight hours later, rats were given a two-bottle test for saccharine preference during the normally scheduled water intake. To ensure that a side preference did not confound the results, half the subjects received water on the left side of the cage, and half received saccharine on the left side of the cage. Moreover, bottle sides were switched 45 min into the testing period.

Experiment 4: Effect of LiCl on overnight water intake—Food was removed, and rats received an ip injection of LiCl (0.15 M, 1.33 ml/100 g body weight; n=5) or vehicle (0.15 M saline, 1.33 ml/100 g body weight; n=5) approximately 30 min prior to lights out, and then water intake was measured for 24 hr.

Experiment 5: Effect of LiCl on AngII-induced water and saline intakes—The procedure for this experiment was identical to that of experiment 1, except that the treatment group received LiCl instead of a GLP-1R agonist. Animals were habituated to *ad libitum* access to both water and 1.5% saline for at least 5 d prior to testing. Approximately 2 h after lights on, food and fluids were removed and rats received a pretreatment injection of LiCl (0.15 M, 1.33 ml/100 g body weight; n=6) or vehicle (0.15 M saline, 1.33 ml/100 g body weight; n=5) ip. Two hours after the pretreatment, rats received an LV injection of AngII (100 ng). Water and saline bottles, but not food, then were made available, and fluid intake was measured for the subsequent two hours. One vehicle-treated and one LiCl-treated rat had total water intake data dropped from analysis due to bottle leakage.

Experiment 6: Effect of LiCl on modified WD-PR-induced water and saline intakes—The procedure for this experiment was identical to that of experiment 2, except that the treatment group received LiCl instead of a GLP-1R agonist. Animals were habituated to *ad libitum* access to both water and 1.5% saline for at least 5 d prior to testing. Rats had fluids removed 24 hr prior to an ip injection of LiCl (0.15 M, 1.33 ml/100 g body weight; n=8) or vehicle (0.15 M saline, 1.33 ml/100 g body weight; n=8). After drug injections, rats were given access to 7 ml water for 2 hr. After the rehydration phase, water and saline, but not food, were returned *ad libitum* and fluid intake was measured for 2 hr.

Data analysis

All data were analysed using Statistica (StatSoft, Tulsa, OK). Water and saline intakes were analysed separately. In Experiment 1, total water and saline intakes were analysed by a one-way repeated measures ANOVA to test for within-subjects effect of Drug. In Experiment 2, total water and saline intakes were analysed with a one-way ANOVA to test for effect of Drug. In Experiment 3, saccharine preference (saccharine intake as percent of total intake) was analysed using a one-way ANOVA to test for effect of Drug. Total water and saline intakes in Experiments 4, 5, and 6 were analysed with a t-test for effect of Drug.

Continuous licks were also recorded during testing periods in Experiments 1, 2, 4, 5, and 6. In Experiments 1, 2, 5, and 6, licks were grouped into 10-min time bins. Although fluid intake was measured for a 2-hr testing period, nearly all intake occurred in the first hour; therefore only lick data from the first hour of the testing period were analysed. For Experiment 1, lick data for water and saline were analysed by a two-way repeated measures ANOVA to test for within-subjects effects of Drug and Time. In Experiments 2, 5, and 6, lick data for water and saline were analysed with a mixed design two-way ANOVA to test for between-subjects effects of Drug and within-subjects effects of Time. Lick data from Experiment 2 were further analysed for lick patterns. Specifically, we used one-way ANOVAs to test for effects of Drug on the number of bursts and on the average number of licks per burst. For these analyses, a burst was defined as a group of at least two licks with a maximum interlick interval of 1 sec. Overnight lick data in Experiment 4 were grouped into 4-hr bins and analysed by a mixed design two-way ANOVA to test for a between-subjects effect of Drug and a within-subjects effect of Time. Statistically significant effects ($p < 0.05$) were further analysed using Newman-Keuls *post-hoc* tests. Drop size in Experiment 5 was analysed with a t-test for effect of Drug.

Results

Experiment 1: Effect of GLP-1R agonists on AngII-induced water and saline intakes

To test the effect of GLP-1R agonists on acutely stimulated fluid intake, liraglutide, exendin-4, or vehicle was injected into the LV before all rats were given a second LV injection of AngII. Analysis of total water intake (Figure 1a) revealed an effect of Drug ($F_{2,24}=17.5$, $p<0.001$). Rats injected with either liraglutide or exendin-4 drank less water than vehicle-treated rats over the 2-h testing period ($p<0.001$ and $p=0.007$, respectively). Furthermore, suppression of water intake was observed when licks for water were analysed (Figure 1b; $F_{10,120}=6.28$, $p<0.001$). *Post-hoc* analysis revealed that the suppression of water intake occurred during the first 10 min of the testing period in liraglutide- or exendin-4-treated subjects (all p -values <0.001 compared to vehicle). In addition, exendin-4-treated rats drank significantly less than liraglutide-treated subjects ($p=0.01$).

There also was an effect of Drug on total 1.5% saline intake (Figure 1c; $F_{2,28}=7.7$, $p=0.002$). *Post-hoc* analysis revealed that exendin-4 treatment led to significantly less saline intake than vehicle- or liraglutide-treated rats ($p=0.005$ and $p=0.002$, respectively); however, there was no effect of liraglutide treatment on total saline intake. In addition, there was an effect of Drug on licks in individual time bins (Figure 1d; $F_{10,140}=3.32$, $p<0.001$). Within the first 10 min of testing, exendin-4-treated rats drank significantly less saline than rats that received liraglutide or vehicle (both p values <0.001).

Experiment 2: Effect of GLP-1R agonists on modified WD-PR-induced water and saline intakes

To test the effects of GLP-1R agonists on fluid intake that is more biased toward saline consumption, we used a modified WD-PR approach. Fluid deprived rats were given injections of liraglutide, exendin-4, or vehicle and then given 7 ml of drinking water before a 2-bottle test of water and 1.5% saline intake. Although water intake is generally low during the testing period of this approach, data analysis revealed that there was an effect of Drug on total water intake (Figure 2a; $F_{2,11}=13.52$, $p=0.001$) and *post-hoc* tests detected that rats treated with either liraglutide or exendin-4 drank less water ($p=0.002$ and $p=0.001$, respectively, compared to vehicle). Furthermore, when binned licks were examined, there was an interaction between Drug and Time (Figure 2b; $F_{10,60}=3.91$, $p<0.001$). The suppression of licks for water by liraglutide or exendin-4 occurred within the first 10 min of testing (p values <0.001 compared to vehicle).

In addition to the observed effects on water intake, drug treatment had an effect on total saline intake (Figure 2c; $F_{2,12}=9.29$, $p=0.004$). Further analysis revealed that either liraglutide- or exendin-4-treated rats drank less saline over the 2-hr testing period than was consumed by vehicle-treated subjects ($p=0.004$ and $p=0.009$, respectively). Analysis of the data in discrete time bins revealed a significant Drug by Time interaction (Figure 2d; $F_{10,60}=4.5$, $p<0.001$). As was observed for water intake, the suppression of saline intake occurred in the first 10 min of testing (p values <0.001).

To determine if the effect of GLP-1R agonists on licks was a function of reduced burst size, burst number, or both, we analysed licking microstructure for water and saline intakes. With respect to water intake, we found an effect of Drug on burst number (Figure 3a; $F_{2,12}=17.42$, $p<0.001$), and *post-hoc* tests found that either liraglutide or exendin-4 decreased the number of bursts for water (both p values <0.001 compared to vehicle). There was, however, no effect on burst size (Figure 3b; $F_{2,6}=1.02$, $p=0.42$). The analysis on burst size was potentially flawed, however, because many animals in both the liraglutide and exendin-4 groups had no bursts, making it impossible to measure burst size. Nevertheless, the effect on burst number was clear.

Similarly, when we examined burst patterns within the licks for saline, we found a significant effect of Drug on burst number (Figure 3c; $F_{2,12}=5.52$, $p=0.02$), but did not detect an effect on burst size (Figure 3d; $F_{2,8}=0.61$, $p=0.57$). *Post-hoc* tests showed that either liraglutide or exendin-4 affected burst number ($p=0.02$ and $p=0.04$ respectively compared to vehicle). Again, the analysis of burst number is questionable because few rats in the liraglutide and exendin-4 groups could be included due to a lack of bursts.

Experiment 3: Does liraglutide (0.05 µg) or exendin-4 (0.05 µg) condition a taste avoidance?

To test the possibility that visceral malaise was responsible for the reduction in fluid intake observed after liraglutide or exendin-4 treatment, we examined whether these doses of GLP-1R agonists would result in avoidance of saccharine when it was paired with the drug. We found a significant effect of Drug on saccharine preference (Figure 4; $F_{3,18}=15.26$, $p<0.001$). *Post-hoc* analysis revealed that exendin-4- or LiCl-treated rats drank significantly less saccharine as a percent of total intake than vehicle-treated rats (both p values <0.001). Furthermore, treatment with exendin-4 or LiCl attenuated the percent of saccharine intake compared to treatment with liraglutide (both p values <0.001). There was, however, no effect of liraglutide on saccharine intake ($p=0.47$).

Experiment 4: Effect of LiCl on overnight water intake

Exendin-4 was found to affect saccharine preference, suggesting that it induces visceral malaise. It was important, however, to test directly the effect of visceral malaise on fluid intake before concluding that this effect of exendin-4 was responsible for the changes in intake. To this end, we determined whether or not LiCl, which is widely used to model visceral malaise, suppressed overnight water intake. An injection of LiCl just before the onset of the dark phase failed to reduce water intake over the subsequent 24 hr (Figure 5a; $p=0.6$). Analysis of intake in 4-hr bins revealed a Drug by Time interaction (Figure 5b; $F_{5,40}=10.03$, $p<0.001$), but *post-hoc* tests showed that the effect was carried by an increase, rather than a decrease, in licking behaviour by the rats given LiCl. This increase occurred during the first 4 hr after drug treatment and no other differences were detected.

Experiment 5: Effect of LiCl on AngII-induced water and saline intakes

To determine if inducing visceral malaise had an effect on stimulated fluid intake, we first examined whether or not treatment with LiCl suppressed AngII-induced water and saline intakes. LiCl had no effect on total water intake (Figure 6a; $p=0.1$), nor did it affect licking for water when the data were analysed in 10-min bins (Figure 6b; $F_{5,45}=1.09$, $p=0.38$). Although it was not statistically significant, we were concerned by the appearance of a mild decrease in 24-hr intake and a mild increase in licks for water. This prompted us to test for differences in volume of water per lick that could explain the apparent, though not statistically significant, difference. Indeed, this analysis revealed that average volume per lick was reduced by LiCl ($p=0.03$). When saline intake was analysed, there was no effect of LiCl on total saline intake (Figure 6c; $p=0.27$), or on binned licks for saline (Figure 6d; $F_{5,45}=0.49$, $p=0.78$).

Experiment 6: Effect of LiCl on modified WD-PR-induced water and saline intakes

Finally, we tested whether or not LiCl treatment affected fluid intake as stimulated by modified WD-PR. There was no effect of Drug on total water intake or on non-cumulative licks for water (Figure 7a & b; $p=0.27$; $F_{5,70}=0.23$, $p=0.95$, respectively). Furthermore, there was no effect of Drug on total saline intake, or on binned licks for saline (Figure 7c & d; $p=0.71$; $F_{5,70}=1.03$, $p=0.41$).

Discussion

The current studies confirm previous reports of GLP-1R-mediated hypodipsia and extend these findings to include the suppression of saline intake. The suppression of fluid intake was observed using two separate paradigms, each of which stimulated both water and hypertonic saline intakes, thereby providing support that the hypodipsic and antinatriorexigenic effects were not paradigm specific. In addition, analysis of licking behaviour indicated that GLP-1R agonists suppressed fluid intake by increasing post-ingestive feedback. Finally, we provided evidence that the hypodipsic and antinatriorexigenic effects were not due to the induction of visceral malaise. Taken together with previous studies demonstrating suppressive effects of GLP-1R agonists on overnight water intake, even when food was unavailable (21), these findings increase support for the concept that GLP-1 is not a feeding-specific satiety signal, but instead leads to a non-specific suppression of intake.

The experiments described used two methods of stimulating fluid intake: icv injection of AngII and the modified WD-PR protocol. The stimulation of fluid intake by AngII is intended to model part, but not all, of the physiological response to hypovolemia. This treatment causes intake of both water and saline solutions (for review see 26). In contrast, the WD-PR protocol is useful to isolate the saline intake effects of a given treatment. Water deprivation stimulates both water and saline intakes, but the WD-PR approach, developed by Sato *et al.* (25), manipulates the availability of the fluids to bias the rat toward saline intake during the testing period. In the present studies, this provided a second evaluation of the saline intake effect of GLP-1R agonist treatment on saline intake, while reducing the confounding variable of water intake. Accordingly, the two approaches provided evidence that GLP-1R agonists suppress both water and saline intakes.

The analysis of lick patterns to evaluate changes in GLP-1R control of drinking behaviour is a novel application of a previously established measure of intake (27, 28). Using this approach, we tested for group differences in burst number (the number of discrete bursts of licks, each with a minimum of two licks separated by less than 1 sec) and found that either liraglutide or exendin-4 decreased the number of bursts for water and for saline over the testing period in the modified WD-PR procedure. We also tested for group differences in burst size (defined as the average number of licks per burst) and found that there was no effect of drug on burst size of licks for water or saline. This lack of an effect, however, may be misleading because the effect of liraglutide or exendin-4 on burst number was so robust that many rats in the drug conditions did not drink at all, and therefore had zero bursts. Rats that did not have any bursts had to be removed from the burst size analysis, thereby decreasing the number of subjects that could be used in the statistical analysis. Therefore, it is possible that the lack of an effect on burst size is due to the small sample size. Accordingly, we urge caution in concluding that GLP-1R agonists do not have an effect on burst size and hope that further studies will provide clarity. Nevertheless, these results collectively suggest that GLP-1R agonists are suppressing water and saline intakes primarily by decreasing the number of bursts. Based on previous studies (for review see 28), this suggests that GLP-1R agonists affected fluid intake by decreasing post-ingestive feedback, rather than by causing changes to orosensory properties of the ingested fluid.

Previous studies suggested subtle differences in the fluid intake effects of liraglutide and exendin-4 (21). Whereas peripheral administration of exendin-4 led to an immediate suppression of water intake, liraglutide had a delayed effect. Consistent with this, the present studies found differences in the response to these two agonists. Specifically, exendin-4, but not liraglutide, suppressed AngII-induced saline intake. Although the molar concentrations of the doses used in these studies are roughly equivalent, there are differences in

pharmacokinetics that may contribute to the observed effects on saline intake. Liraglutide resists enzymatic breakdown through self-aggregation and albumin binding, whereas exendin-4 is not susceptible to enzymatic degradation and is cleared from the body through glomerular filtration (for review see 24, 29). These and other differences may have contributed to the observed responses, but future studies are needed for a precise explanation. Nevertheless, the suppression of saline intake by liraglutide in the WD-PR studies and the suppression of water intake by both agonists in both studies highlights the fluid intake relevance of the GLP-1 system.

GLP-1R agonists have been shown to condition a taste avoidance and cause pica, raising concern that the food intake suppression is secondary to a more primary visceral malaise (22). This concern is perpetuated by reports of humans experiencing nausea after liraglutide or exendin-4 injections (30, 31). This effect, however, is most likely dose- and route of administration-dependent. Because we were unable to find any tests of conditioned taste avoidance using lateral ventricle injections of the dose used in our studies, it became important for us to test the specific parameters for our studies. When we paired an injection of exendin-4 with saccharine, it led to a robust avoidance of the saccharine solution 48 hr later, suggesting that the present dose of exendin-4 produced visceral malaise. This raised the concern that the hypodipsic and antinatriorexigenic effects were secondary to nausea. We reject this conclusion, however, for several reasons. First, liraglutide failed to condition a taste avoidance to saccharine even though the same dose of liraglutide suppressed water intake in Experiment 1 and both water and saline intakes in Experiment 2. Second, studies have shown that GLP-1R-mediated suppression of intake is separable from the induction of nausea. For example, microinjections of GLP-1R agonists into the PVN suppress food intake without conditioning a taste avoidance, whereas injections into the central nucleus of the amygdala generate learned taste avoidance with no effect on food intake (32, 33). Third, and perhaps most important, the relationship between visceral malaise and fluid intake appears to be less straightforward than the clear association between nausea and hypophagia. Accordingly, further evaluation was needed.

To determine if visceral malaise was a primary factor in the suppression of fluid intake induced by GLP-1R agonists, we examined whether injections of LiCl, which is well known to cause visceral malaise (34, 35), had a similar effect on fluid intake under the conditions used in Experiments 1 and 2. The logic of this approach is straightforward: if the suppression of fluid intake by GLP-1R agonists was because of visceral malaise, then inducing visceral malaise should similarly suppress fluid intake. When we injected rats with LiCl just before lights off, we found an effect of LiCl treatment on overnight water intake, but the effect was in the opposite direction of that observed after GLP-1R agonist injections. Specifically, within the first 4 hr after LiCl injection, rats exhibited a robust increase in water intake. The nature of the hyperdipsic effect is unclear, and could be mediated by many physiological responses to LiCl, but is nevertheless in stark contrast to the decreased overnight intake observed after injection with GLP-1R agonists (21). Furthermore, LiCl administration did not suppress water or saline intake in our tests of AngII- or WD-PR-induced fluid intake. Accordingly, it is logical to conclude that any nausea or nausea-like symptoms generated by exendin-4 are not the primary cause of its hypodipsic and antinatriorexigenic effect. The present findings are consistent with previous reports that describe no suppression of fluid intake after LiCl injection (36–38). These previous studies used doses or paradigms that were otherwise not applicable, making our experiments particularly important. Our findings do not discount previous evidence that GLP-1 is involved in the detection of visceral malaise. Indeed, GLP-1 appears to be an important part of that detection. LiCl injections induce c-Fos expression in GLP-1 positive cells, and GLP-1R antagonists block LiCl-induced hypophagia, CTA, and pica (39). Our studies do,

however, suggest that this role of GLP-1 is separable from its suppressive effects on fluid intake because nausea or malaise do not reliably decrease fluid intake.

Conclusions drawn from the present results should consider the physiological effects of GLP-1R activation that may influence body fluid homeostasis. For instance, GLP-1R agonists increase diuresis and natriuresis (11, 20). This effect is unlikely to contribute to the observed effects, however, because any loss of water or NaCl would lead to increased, rather than decreased, fluid intake. Likewise, GLP-1 enhances glucose-stimulated insulin release, but insulin increases water intake (40), which makes this another unlikely explanation for the present results. A more plausible possibility is that the observed effects are related to the pressor response of GLP-1R agonists (41). Increases in blood pressure suppress water intake (42), but careful examination of the relevant literature reveals that GLP-1R-mediated increases in blood pressure are more transient than its effects on water intake. Specifically, blood pressure returns to normal 2.5 hr after a peripheral injection of exendin-4, but water intake is suppressed 3 hr after a similar dose of exendin-4 (21, 43). Therefore, these explanations, although important to consider, do not appear to underlie the observed fluid intake suppression by GLP-1R agonists.

The present studies used ventricular administration of the GLP-1R agonists. As such, the precise site of action for the hypodipsic and antinatriorexigenic effects of GLP-1R agonists remains unknown. There are, nevertheless, several identified structures that mediate the feeding effects of GLP-1R. These structures include the paraventricular nucleus of the hypothalamus, the lateral hypothalamus, the dorsomedial hypothalamus, the ventromedial hypothalamus, and the NTS (14, 44–46). Although these structures are clearly important for the feeding effects of GLP-1R agonists, it is an open question whether or not the hypodipsic and antinatriorexigenic effects are mediated similarly. In addition to the areas implicated in the feeding effects, GLP-1-containing fibers and GLP-1R expression is found in other structures, most notably in areas that influence body fluid homeostasis. These brain areas include the anteroventral third ventricle region and the subfornical organ (4, 47). Future studies are needed to determine the requisite neural substrates for the observed effects on water and saline intakes.

The finding that the ingestive effects of GLP-1R agonists are not specific to food intake makes it tempting to speculate that GLP-1 affects regions more generally involved in motivated behaviours. In this respect, research focused on the nucleus accumbens may be particularly important. Injections of GLP-1 directly into the nucleus accumbens core suppressed food intake, and administration of the GLP-1R antagonist exendin-9 induced hyperphagia (45). Furthermore, recent studies report that the glucagon-like peptide 1 analogue Exendin-4 attenuates alcohol mediated behaviours in rodents (48). As such, the present findings add to a growing literature indicating that GLP-1R agonists are involved in a more general suppression of motivated behaviours.

In summary, we found hypodipsic and antinatriorexigenic effects of GLP-1R agonists, and lick pattern analysis suggests that these effects were mediated by a change in post-ingestive feedback. Furthermore, it is unlikely that the suppressive effect of the agonists on water or saline intake was primarily due to visceral malaise because injection of LiCl did not similarly decrease water or saline intakes. Taken together, these data extend what is known about GLP-1 and fluid intake to show a novel effect on saline intake and the results provide a challenge to the hypothesis that GLP-1R agonists have a selective effect on food intake.

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Figure 1.

Effect of liraglutide (0.05 μ g) or exendin-4 (0.05 μ g) on AngII-induced fluid intake. **(a)** Treatment with either liraglutide or exendin-4 suppressed total water intake during the 2-hr test after AngII injections. **(b)** The suppression of noncumulative licks by liraglutide or exendin-4 occurred during the first 10 min of testing. **(c)** Treatment with exendin-4, but not liraglutide, suppressed 1.5% saline intake. **(d)** Suppression of noncumulative licks for 1.5% saline by exendin-4 occurred during the first 10 min of testing. Asterisks indicate differences from vehicle ($p < 0.05$), pound indicates difference from liraglutide ($p < 0.05$).

Figure 2.

Effect of liraglutide (0.05 μ g) or exendin-4 (0.05 μ g) on WD-PR-induced fluid intake. (a) Treatment with either liraglutide or exendin-4 suppressed total water intake during the 2-hr test after WD-PR. (b) The suppression of noncumulative licks by liraglutide or exendin-4 occurred during the first 10 min of testing. (c) Treatment with either exendin-4 or liraglutide suppressed total 1.5% saline intake. (d) Suppression of licks for 1.5% saline occurred during the first 10 min of testing. Asterisks indicate differences from vehicle ($p < 0.05$).

Figure 3.

Effect of liraglutide (0.05 µg) or exendin-4 (0.05 µg) on licking behaviour during WD-PR two-bottle testtesting period. (a) Liraglutide- or exendin-4-treated rats exhibited fewer licking bursts for water during the 2 hr test. (b) Neither liraglutide nor exendin-4 affected saline burst size. (c) Similar to the effect on water, either liraglutide or exendin-4 suppressed saline burst number. (d) Neither liraglutide nor exendin-4 affected saline burst size. Asterisks indicate differences from vehicle ($p < 0.05$).

Figure 4.

Preference test after a single pairing of saccharine and liraglutide (0.05 μ g), exendin-4 (0.05 μ g), LiCl (0.15 M, 1.33 ml/100 g body weight), or vehicle (0.9% saline). Rats given exendin-4 or LiCl immediately after saccharine exposure showed a robust avoidance of 0.1% saccharine 2 d later. Liraglutide, however, did not condition a taste avoidance. Asterisks indicate differences from vehicle ($p < 0.05$), pound indicates difference from liraglutide ($p < 0.05$).

Figure 5.

Effect of LiCl (0.15 M, 1.33 ml/100 g body weight) on 24 hr water intake. **(a)** Injections of LiCl did not have an effect on total 24 hr water intake. **(b)** Examination of noncumulative licks for water in 4-hr bins revealed that rats treated with LiCl had a robust increase in licks during the first 4 hr after drug injections. There were, however, no other differences between groups during the remainder of the 24 hr. Asterisk indicates differences from vehicle ($p < 0.05$).

Figure 6.

Effect of LiCl (0.15 M, 1.33 ml/100 g body weight) on AngII-induced fluid intake. **(a)** There was no effect of LiCl on total water intake during the 2-hr test after AngII injections. **(b)** There was no effect of LiCl on noncumulative licks for water when 10-min bins were examined. **(c)** There was no effect of LiCl on total 1.5% saline intake during the 2-hr test after AngII injections. **(d)** There was no effect of LiCl on noncumulative licks for saline when 10-min bins were examined.

Figure 7. Effect of LiCl (0.15 M, 1.33 ml/100 g body weight) on WD-PR-induced fluid intake. **(a)** There was no effect of LiCl on total water intake during the 2-hr test after WD-PR. **(b)** There was no effect of LiCl on noncumulative licks for water when 10-min bins were examined. **(c)** There was no effect of LiCl on total 1.5% saline intake during the 2-hr test after WD-PR. **(d)** There was no effect of LiCl on noncumulative licks for saline when 10-min bins were examined.