GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS SUPPRESS WATER INTAKE INDEPENDENT OF EFFECTS ON FOOD INTAKE

Running header: GLP-1 and water intake

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Abstract

Glucagon-like peptide-1 (GLP-1) is produced by and released from the small intestine following ingestion of nutrients. GLP-1 receptor (GLP-1R) agonists applied peripherally or centrally decrease food intake and increase glucose-stimulated insulin secretion. These effects make the GLP-1 system an attractive target for the treatment of type-2 diabetes mellitus and obesity. In addition to these more frequently studied effects of GLP-1R stimulation, previous reports indicate that GLP-1R agonists suppress water intake. The present experiments were designed to provide greater temporal resolution and site specificity for the effect of GLP-1 and the long-acting GLP-1R agonists, exendin-4 and liraglutide, on unstimulated water intake when food was and was not available. All three GLP-1R ligands suppressed water intake after peripheral (IP) administration both in the presence of and absence of food; however, the magnitude and time frame of water intake suppression varied by drug. GLP-1 had an immediate, but transient, hypodipsic effect when administered peripherally, whereas the water intake suppression by IP exendin-4 and liraglutide was much more persistent. Additionally, intracerebroventricular administration of GLP-1R agonists suppressed water intake when food was absent, but the suppression of intake showed modest differences depending on whether drug was administered to the lateral or fourth ventricle. To the best of our knowledge, this is the first demonstration of GLP-1 receptor agonists affecting unstimulated, overnight intake in the absence of food, the first test for anti-dipsogenic effects of hindbrain application of GLP-1 receptor agonists, and the first test of a central effect (forebrain or hindbrain) of liraglutide on water intake. Overall, these results show that GLP-1R agonists have a hypodipsic effect that is independent of GLP-1R-mediated effects on food intake, and this occurs, in part, through CNS GLP-1R activation.

Key words: GLP-1, exendin-4, liraglutide, hypodipsia, thirst
Introduction

Glucagon-like peptide-1 (GLP-1) is produced primarily by L-cells in the intestine and neurons in the nucleus of the solitary tract (NTS) of the hindbrain in response to nutrient entry in the gastrointestinal (GI) tract (22, 27, 28). Administration of GLP-1, or GLP-1 receptor (GLP1-R) agonists, decreases food intake, inhibits gastric emptying, and enhances the production of insulin in response to glucose (11, 23). The role of GLP-1 as an incretin hormone has made the GLP-1 system an attractive treatment strategy for type-2 diabetes mellitus (T2DM); however, the rapid enzymatic metabolism of the endogenous peptide limits its half-life to approximately 2 minutes (14), thus making GLP-1 clinically impractical. Accordingly, GLP-1R agonists with increased resistance to rapid enzymatic degradation have been developed. Two examples currently used clinically for T2DM treatment are exendin-4, with a half-life of about 2.5 hr, and liraglutide, which has a half-life of approximately 13 hr (29). Interestingly, these same GLP-1R agonists are also potential treatments for obesity due to the potent suppressive effects on food intake and body weight that have been reported in both humans and animal models (2, 25, 26, 36, 43).

A small number of studies also show a role for GLP-1R ligands in drinking behavior in both rats and humans (9, 21, 30, 40, 45). These studies, however, did not sufficiently determine if the observed effects on drinking were more directly related to the suppressive effects on feeding, especially with respect to unstimulated water intake. This is of critical importance because water and food intakes often occur together. Indeed, most of the daily water intake in rats occurs during bouts of feeding (7). Accordingly, treatments that reduce food intake also are likely to decrease water intake, making it difficult to determine from these earlier studies if GLP-1R agonists have an effect on water intake, independent of any effects on food intake.

In the present experiments, we tested the effect of three GLP-1R ligands (GLP-1, exendin-4, and liraglutide) on 24 hr water intake in the presence or absence of food. Drugs were administered peripherally (IP) or centrally (lateral or fourth ventricle) to evaluate the potential for a forebrain and/or hindbrain central site of action. Moreover, automated intake measures provided detailed and novel temporal and ligand specific characteristics of the effects of the GLP-1R agonists on water intake. To the
best of our knowledge, this is the first test of GLP-1 receptor agonists on unstimulated, overnight intake
in the absence of food, the first test for anti-dipsogenic effects of hindbrain application of GLP-1 receptor
agonists, and the first test of a central effect (forebrain or hindbrain) of liraglutide on water intake.
Collectively, the results are consistent with the hypothesis that GLP-1R agonists produce a potent
hypodipsic effect independent of any effects on food intake suppression after either peripheral or central
administration.

Methods

Animals

Forty-four adult male Sprague-Dawley rats (325-349 g at time of purchase; Harlan Laboratories
Inc., Indianapolis, IN) were used for the experiments. Animals were housed individually in hanging
stainless steel wire mesh cages (Unifab Corporation, Kalamazoo, MI) in a temperature and humidity
controlled room and maintained on a reverse 12:12 hr light:dark schedule (lights off at 10:00 AM and on
at 10:00 PM). Standard rodent chow and tap water were available ad libitum except where noted. A
repeated measures design was used within each experiment, but animals were not used in multiple
experiments. 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.

ICV cannula implantation and placement verification

Rats in Experiments 3 and 4 were implanted with chronic indwelling ICV cannulae aimed at the
lateral or fourth cerebral ventricle, respectively. Rats were anesthetized 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. A small burr hole was drilled
and guide cannulae (26 ga; Plastics One Inc., Roanoke, VA) were implanted using the following
coordinates: lateral ventricle (LV), 0.9 mm posterior to bregma, 1.4 mm lateral to midline, and 1.8 mm
ventral to dura; 4V, 2.5 mm anterior to the occipital structure, on midline, and 4.8 mm ventral to skull. Cannulae were affixed to the skull with bone screws and dental cement and rats were given a single postoperative injection of carprofen (5 mg/kg, IM; Pfizer Animal Health, New York, NY). At least 5 days after surgery, accurate cannula placement was verified by the response to injection of 10 ng angiotensin II (LV) or 210 µg 5-thio-D-glucose (4V). Rats that drank at least 6 ml of water in 30 min after angiotensin II or that had at least a 2-fold increase in blood glucose after 5-thio-D-glucose were included in subsequent testing. All animals were verified again using the same protocol after the last experimental day and data from any rat that did not meet verification requirements were removed from the analysis.

**Drug injections and intake measures**

Injections were given approximately 30 min before lights out. Peripheral injections were all IP and central injections were made with a 33 ga injection cannula inserted into PE 50 tubing attached to a 2 µl Hamilton syringe. Injection cannulae were left in place for approximately 1 min after each injection. Water bottles were weighed and returned to the cages immediately after each injection. Total water intake during the testing period was calculated by the difference in pre- and post-test water bottle weight and the distribution of intake was assayed by counting licks in 20 min bins using 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 was processed in a MATLAB (MathWorks, Natick, MA) software environment before being ported to excel for final analysis. Water 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. Food hoppers were measured before and after food intake tests. Spillage was collected on a plastic transparency under each cage and was included in the measures as uneaten food.

**Experiment 1: Peripheral administration of GLP-1R agonists on water and food intake**
Using a repeated measures design, rats (n=12) were injected (IP) with GLP-1(7-36) (500 μg/kg; American Peptide, Sunnyvale, CA), exendin-4 (3μg/kg; American Peptide, Sunnyvale, CA), liraglutide (100 μg/kg; Bachem, Torrance, CA), or vehicle (1 ml/kg 0.9% saline). The doses were selected based on previous studies showing that each is within the effective range for a hypophagic response following IP administration (12, 46). Food and water intakes were measured over the subsequent 24 hr. Each rat received each treatment condition in a partial Latin squares design with each testing day separated by 3-4 d.

Experiment 2: Peripheral administration of GLP-1R agonists on water intake in the absence of food

The procedures employed in Experiment 1 were repeated using the same number of rats and the same injection protocol except that food was removed immediately before the injections and remained unavailable for 24 hr, during which water intake was measured. Food was returned after the 24-hr drinking test.

Experiment 3: Lateral ventricle administration of GLP-1R agonists on water intake in the absence of food

Experiment 3 was identical to Experiment 2, but drugs were injected into the LV. Ten rats received injections of GLP-1 (10 μg), exendin-4 (0.1 μg), liraglutide (2 μg), or vehicle (0.9% saline), doses that reliably suppress food intake (13, 46)(Unpublished pilot work). All injections were 1 μl. A partial Latin squares design was used with 4-6 d between treatments.

Experiment 4: Fourth ventricle administration of GLP-1R agonists on water intake in the absence of food

Experiment 4 was identical to Experiment 3 except drugs were injected into the 4V.

Statistical analysis

All data were analyzed using Statistica (StatSoft, Tulsa, OK). For each experiment a two-way repeated measures ANOVA was used to test for within-subjects effects of Drug and Time. When there
were more than 4 levels in any factor (e.g., 12 time bins), a Greenhouse-Geisser correction was used to
determine statistical significance. Dark phase (12-hr) water intake, 24-h water intake, and 24-h food
intake were each analyzed by repeated measures one-way ANOVA. Significant main or interaction
effects ($p<0.05$) were further analyzed using Newman-Keuls post hoc tests.

Results

Experiment 1: Peripheral administration of GLP-1R agonists on water and food intake.

Analysis of the cumulative and noncumulative water intake revealed that the GLP-1R agonists
each suppressed water intake, but the magnitude and time course of suppression was drug specific (Figure
1). GLP-1 and exendin-4 rapidly suppressed water intake, whereas the effect of liraglutide was slower to
develop. Additionally the effect of GLP-1 was transient compared to the longer-lasting effect of exendin-
4 or liraglutide. A two-way repeated measures ANOVA on noncumulative intake in 4-hr bins revealed
main effects of Drug ($F_{1.95, 21.44}=49.16$, $p<0.001$), Time ($F_{2.37, 26.06}=111.23$, $p<0.001$), and a Drug x Time
interaction ($F_{4.74, 52.19}=34.96$, $p<0.001$; Figure 1a). Post hoc tests confirmed a statistically significant
suppression of water intake in the first 4 hr after exendin-4 administration ($p<0.001$ compared to vehicle).
This immediate suppression was followed by elevated intake during the final 4-hr bin (20 hr through 24
hr), during which water intake was greater than that observed by the vehicle group ($p<0.05$). Liraglutide
suppressed water intake during the first 4-hr bin compared to vehicle treatment ($p<0.001$), but this
suppression was not as large as that observed after exendin-4 treatment ($p<0.001$). The suppressive effect
of liraglutide became increasingly robust throughout the first 12 hr of testing. No differences in water
intake were observed during the majority of the subsequent light phase, which began during hour 12, but
water intake was low during this period in all groups. Similar to exendin-4, rats treated with liraglutide
also drank more water during the last 4 hr of testing. Indeed, water intake by rats treated with liraglutide
was greater than all other groups in the last 4-hr bin (all $p$-values<0.01).

Analysis of cumulative intake largely mirrored the noncumulative data, but provides an
additional, perhaps clearer illustration that the decrease of water intake after liraglutide and exendin-4 was
maintained throughout the 24 hr period (Figure 1b). A two-way repeated measures ANOVA on the cumulative 4-hr data confirmed main effects of Drug ($F_{1.87, 20.52}=73.78$, $p<0.001$), Time ($F_{1.66, 18.22}=410.44$, $p<0.001$), and a Drug x Time interaction ($F_{3.97, 43.64}=49.26$, $p<0.001$) and post hoc tests showed that the suppressive effect of exendin-4 and liraglutide was statistically significant by the end of the first 4 hr.

Water intake after liraglutide or exendin-4 injection remained lower at every time during the testing period ($p<0.001$ and $p<0.001$, respectively) compared to intakes after vehicle injection. This indicates that at the end of the 24-hr test, the animals that received liraglutide and exendin-4 did not fully compensate for the initial suppression of water intake.

As expected, the vast majority of water intake occurred during the 12-hr dark phase. Analysis of dark phase intake (0-12 hr) confirmed the findings described above. Specifically, a repeated measures ANOVA detected a main effect of Drug ($F_{3,33}=83.37$, $p<0.001$), and post hoc tests confirmed that both exendin-4 and liraglutide suppressed total water intake during the dark phase ($p<0.001$ and $p<0.001$ compared to vehicle; Figure 1c).

Although the effect of GLP-1 was not apparent in the above analyses, a transient effect of GLP-1 was detected when data were analyzed in shorter time bins (Figure 1d). When 24-hr intake was broken into 1-hr bins, ANOVA confirmed main effects of Drug ($F_{1.95, 21.44}=49.16$, $p<0.001$), Time ($F_{5.38, 59.21}=19.55$, $p<0.001$), and a Drug x Time interaction ($F_{8.51, 93.56}=6.93$, $p<0.001$). Post hoc tests confirmed a transient effect of GLP-1 that was limited to the first hour after treatment and is illustrated in Figure 2. The initial effect of GLP-1 disappeared quickly, and no further effects of GLP-1 on water intake were observed.

As expected, exendin-4 and liraglutide reliably decreased 24-hr food intake, but we did not find a 24-hr hypophagic response after GLP-1 administration (Figure 2a). Analysis of the 24-hr food intake revealed a main effect of Drug (repeated measures ANOVA, $F_{3.33}=121.65$, $p<0.001$). Post hoc tests revealed that rats given exendin-4 or liraglutide ate less than rats in the vehicle ($p<0.001$; $p<0.001$ respectively) or GLP-1 groups ($p<0.001$; $p<0.001$ respectively). Moreover, the magnitude of suppression by liraglutide was greater than by exendin-4 treatment ($p<0.001$).
The availability of food during the water intake measures made it impossible for us to estimate if the observed effect on water intake was independent of the effect on food intake. Indeed, the percent suppression in 24-hr water intake was remarkably similar to percent suppression in 24-hr food intake within each drug condition (Figure 2a,b). Moreover, when the data were expressed as a ratio of water to food, intake differences were neutralized ($F_{3,33}=0.94$, $p=0.43$; Figure 2c), making it necessary to test if the effect on water intake was secondary to an effect on food intake.

**Experiment 2: Peripheral administration of GLP-1R agonists on water intake in the absence of food.**

To test the possibility that the observed hypodipsia was secondary to the suppression of food intake, we repeated the experiment but removed food immediately before drug administration. The absence of food did not, however, prevent the anti-dipsogenic effects of GLP-1R agonists and the direction, timing, and magnitude of suppression were remarkably similar to what we observed in the previous experiment (Figure 3). Analysis of noncumulative, 4-hr binned water intake confirmed main effects of Drug ($F_{2.08, 22.90}=5.19$, $p<0.05$), Time ($F_{1.46, 16.10}=25.36$, $p<0.001$) and a Drug x Time interaction ($F_{6.03, 66.37}=14.91$, $p<0.001$; Figure 3a). Similar to the previous results, exendin-4 administration quickly and robustly suppressed water intake ($p<0.001$ vs vehicle). After the initial four hours, intake was similar to controls for all of the remaining bins except the last, during which there was again a moderate but statistically significant increase in water intake ($p<0.01$ compared to vehicle). The effect of liraglutide on water intake was also similar to that observed in the previous experiment. Suppression of water intake did not occur immediately after drug administration, but was observed in the second and third 4-hr bins ($p<0.001$ compared to vehicle in the 4-8-hr bin; $p<0.001$ compared to vehicle in the 8-12-hr bin). Rats given liraglutide showed a moderate, but statistically significant increase in water intake during the final 4-hr bin ($p<0.001$ compared to vehicle).

Analysis of cumulative water intake confirmed the robust hypodipsia induced by peripheral exendin-4 and liraglutide (Figure 3b). A two-way repeated measures ANOVA found main effects of Drug ($F_{1.91, 21.04}=10.17$, $p<0.001$), Time ($F_{1.06, 11.63}=36.65$, $p<0.001$), and a Drug x Time interaction ($F_{2.96, 32.70}=5.04$, $p<0.01$).
By examining the data in this way it is evident that animals that received liraglutide maintained a decrease in overall water intake throughout the entire 24 hr (p<0.05 compared to vehicle at 4 hr; p<0.001 compared to vehicle at all other time points). Rats that received exendin-4, however, increased intake near the end of the test (hrs 20-24), thereby eliminating cumulative 24-hr differences from controls.

Dark phase water intake again was examined and a repeated measures ANOVA confirmed a main effect of Drug (F3,33=13.62, p<0.001; Figure 3c). Post hoc tests revealed a suppressive effect of liraglutide, but GLP-1 and exendin-4 were not significantly different from vehicle. Although this analysis did not detect an effect from exendin-4, the analysis of 4-hr binned and cumulative intake clearly indicated that GLP-1R agonists suppressed water intake in the absence of food.

Similar to the results of Experiment 1, when we probed the water intake data by examining 1-hr binned intake, the main effects of Drug (F2.08, 22.90=5.19, p<0.05), Time (F4.44, 48.89=9.68, p<0.001), and a Drug x Time interaction (F6.59, 72.53=3.98, p<0.01) persisted (Figure 3d). Both the cumulative and noncumulative data show a transient, but robust suppression of water intake by GLP-1 (p<0.05 compared to vehicle at 4 and 8 hr in the cumulative data; p<0.001 compared to vehicle the first bin in noncumulative data). It is likely that the prolonged effect apparent in the cumulative analysis was due to the large magnitude of intake suppression during the first hour after drug administration. Accordingly, the findings from Experiment 2 demonstrate that the anti-dipsogenic effect of these GLP-1R agonists did not depend on a more proximal effect on food intake.

Experiment 3: Lateral ventricle administration of GLP-1R agonists on water intake in the absence of food

To evaluate the role of central GLP-1R in the observed effects on water intake, we made injections of GLP-1R agonists into the LV. Analysis of noncumulative or cumulative data confirmed that LV injections of liraglutide and exendin-4, but not GLP-1 affected water intake in the absence of food (Figure 4). Overall the effects on water intake were similar to those observed after peripheral drug administration, except that we did not find an effect of GLP-1 on water intake. Repeated measures
ANOVA on noncumulative, 4-hr binned intake confirmed a main effect of Drug (F2.18, 19.60=6.81, p<0.01),
time (F1.56, 14.00=9.22, p<0.01) and a Drug x Time interaction (F2.05, 18.49=6.59, p<0.01; Figure 5a). Rats
given exendin-4 drank moderately more than controls during the first 4-hr bin (p<0.01 compared to
vehicle); however, water intake after LV vehicle delivery was considerably lower during this bin than that
observed in Experiments 1, 2, and 4, suggesting that this increase by exendin-4 is likely an artifact of an
abnormally low intake by vehicle-treated rats during this bin rather than being due to drug-induced
hyperdipsia. The suppressive effect of exendin-4 and liraglutide was, however, reliable and clear during
the next two 4-hr bins (p<0.001 exendin-4 or liraglutide compared to vehicle 4-8-hr bin; p<0.001 exendin-
4 compared to vehicle 8-12-hr bin; p<0.01 liraglutide compared to vehicle 2-12-hr bin). Unlike our
analysis of the intake after peripheral injections, there was no compensatory drinking in the 20-24 hr
period after drug administration. Evaluation of 1-hr intake bins did not reveal any transient differences in
intake after GLP-1 administration (Figure 4d).

Analysis of the cumulative intake highlighted that exendin-4 and liraglutide reduced overall water
intake throughout the 24-hr testing period (Figure 4b). Repeated measures ANOVA revealed main effects
of Drug (F1.99, 17.91=5.31, p<0.05), time (F1.18, 10.58=35.71, p<0.001), and a Drug x Time interaction (F2.25,
20.27=12.50, p<0.001). Post hoc testing found significant differences between the liraglutide and control
group beginning 8 hr after drug administration that persisted through the remainder of the testing (p<0.05
compared to vehicle at 8 hr; p<0.001 compared to vehicle at all subsequent time points). Analysis of
cumulative water intake found that the decreased intake after exendin-4 was not significant until hr 12
(p<0.001 compared to vehicle at 12-24 hr). Both the cumulative and noncumulative data show a clear
anti-dipsogenic effect after LV injection of exendin-4 or liraglutide that did not depend on a concurrent
hypophagia.

Dark phase intake is shown in Figure 4c and a repeated measures ANOVA confirmed a main
effect of Drug (F3.27=70.26, p<0.001). Similar to Experiments 1 and 2, exendin-4 and liraglutide both
suppressed total 12-hr water intake to a similar extent, but there was no effect of GLP-1 (p<0.01; p<0.01;
p=0.45 respectively compared to vehicle).
Experiment 4: Fourth ventricle administration of GLP-1R agonists on water intake in the absence of food

GLP-1 receptor agonists also were injected into the 4V to evaluate mediation of water intake suppression by hindbrain substrates. Analysis of noncumulative water intake revealed a main effect of Drug ($F_{1.75, 15.78}=4.13$, $p<0.05$), Time ($F_{2.94, 26.48}=9.55$, $p<0.001$), and a Drug x Time interaction ($F_{4.70, 42.33}=6.54$, $p<0.001$; Figure 5a). Again, after exendin-4 administration there was robust suppression of water intake during the second 4-hr bin ($p<0.001$ vs vehicle) and third 4-hr bin ($p<0.001$ vs vehicle). There was also suppression of water intake after liraglutide administration during the second 4-hr bin ($p<0.001$ vs vehicle); however, this suppression appeared to be neither as robust, nor as long lasting, as that observed after LV administration. Similar to the results from LV drug administration there was no effect of GLP-1 on water intake in any bin, nor was there a statistically significant increase in water intake during the last bin of the testing period. Analysis of data in 1-hr bins failed to reveal any additional effects (Figure 5d).

Analysis of cumulative water intake confirmed the effect of liraglutide and exendin-4, and revealed significant suppression of intake by GLP-1 (Figure 5b). A two-way repeated measures ANOVA found a main effect of Drug ($F_{1.69, 15.17}=6.33$, $p<0.05$), Time ($F_{1.52, 13.69}=44.93$, $p<0.001$), and a Drug x Time interaction ($F_{2.61, 23.45}=9.08$, $p<0.001$). Like the noncumulative analysis, both liraglutide and exendin-4 decreased water intake at 8 hr, however, here it is also evident that cumulative intake remained lower then vehicle intake throughout the rest of the testing period ($p<0.001$ for either liraglutide or exendin-4 compared to vehicle at all time points). Cumulative representation of the data also revealed that GLP-1 did have an effect on water intake. Post hoc testing found significant differences between the GLP-1 and control group beginning 8 hr after drug administration that persisted through the remainder of the 24 hr ($p<0.001$ compared to vehicle at 8-24hr time points).

Analysis of dark phase intake also detected a suppressive effect of all three GLP-1R agonists after 4V administration (Figure 5c). A repeated measures ANOVA confirmed a main effect of Drug ($F_{3,27}=137.91$, $p<0.001$) and post hoc tests showed similar hypodipsia in GLP-1- or liraglutide-treated rats
(p<0.05 and p<0.01 respectively compared to vehicle), and greater suppression of intake after exendin-4 administration (p<0.001 compared to vehicle, p<0.05 compared to GLP-1, p<0.05 compared to liraglutide). Overall the data from the central injections suggest that both forebrain and hindbrain sites are involved in GLP-1R agonist induced hypodipsia.

**Discussion**

The current findings provide a comprehensive evaluation of the time-course of the hypodipsic effects of GLP-1R agonists and provide evidence that suppression of water intake occurred independent of any effects on food intake. Either peripheral or central administration of GLP-1R agonists, at doses that produce hypophagia (12, 13, 46), suppressed water intake, even in the absence of food. Moreover, to the best of our knowledge, this is the first test of water intake after hindbrain injection of these GLP-1R ligands, and the first time that this effect has been reported after either forebrain or hindbrain administration of liraglutide. The consistent doses used in the LV and 4V injections provide the first comparison of the efficacy of forebrain and hindbrain routes of administration and highlight the potential for a hindbrain site of action in the anti-dipsogenic response to GLP-1R agonists.

Peripheral administration of GLP-1, exendin-4, or liraglutide suppressed unstimulated overnight water intake in rats both in the presence and absence of food. The pattern of water intake suppression after peripheral injection was consistent with the known half-lives of the drugs tested in these studies (29). Specifically, GLP-1, which is very rapidly degraded, only suppressed water intake during the first hour of testing, whereas the effect of exendin-4 persisted throughout the first four hours of testing. Furthermore, liraglutide, which has the longest half-life of the drugs tested, had the longest-lasting effect on water intake, with hypodipsia maintaining for 12 hr after IP injection. Therefore, it is reasonable to conclude that differences in the time frame of the hypodipsic response to peripheral injections were largely related to the stability of the ligands.

Hypodipsia was also evident after central administration of GLP-1R agonists, although the pattern of intake was slightly different compared to that observed following peripheral delivery. These
differences may provide important information about the site(s) and mechanism(s) of action for the different agonists. Following peripheral injection, liraglutide was more potent at suppressing water intake than was exendin-4. The relative potency of exendin-4 and liraglutide differed, however, when the drugs were injected centrally. Exendin-4 and liraglutide produced similar suppression of intake after LV injections, but exendin-4 was more effective than liraglutide after 4V injection. The different doses used could be partly responsible for the differences between the responses to peripheral and central injections, but it is notable that the direction of the difference was not the same after LV or 4V injection, making it unlikely that it was simply a dose-related artifact. The differences after IP, LV, and 4V injections make it tempting to speculate that an interaction between the mechanism of action of the drugs and the route of administration was responsible. Nevertheless, the hypodipsic effect of GLP-1 agonists after central administration was clear; suggesting that at least part of the effect was mediated by central substrates, similar to studies done on food intake (16).

The comparison of LV and 4V administration revealed differences in the efficacy of GLP-1 that depended on the site of injection. Analysis of cumulative, as well as dark phase intake, revealed an effect of GLP-1 after 4V, but not after LV, administration. The apparent differences in intake after LV or 4V administration of vehicle may, however, contribute to this effect. We did not find any differences in the response to LV or 4V exendin-4; injection into either ventricle potently suppressed water intake. Concluding that there was no difference based on site of administration is difficult, however, because intake was almost completely eliminated between the 4th and 12th hour of testing, creating the possibility of a floor effect. Lastly, liraglutide suppressed water intake, but the magnitude and pattern of the suppression differed based on injection site. The effect of liraglutide was somewhat more robust when administered into the LV than when it was injected into the 4V, raising the possibility of additional forebrain sites at which GLP-1R agonists can act to suppress water intake. Regardless of these nuances in the response, the data clearly demonstrate suppression of water intake by exendin-4 or liraglutide and strongly implicate the hindbrain as a site of action, with the potential for additional receptive sites in the forebrain.
The precise mechanisms underlying peripheral or central GLP-1R-mediated hypodipsia remains to be determined. Meal size suppression following intraperitoneal administration of GLP-1 has been shown to depend upon vagal afferent signaling (34). Vagal afferents synapse on NTS neurons, which in turn connect to a network of structures including the amygdala, PVN, lateral hypothalamus, and parabrachial nucleus (22, 31, 32). Although these connections have been implicated in the control of food intake, their specific relevance to an effect on water intake remains to be determined, especially with relevance to GLP-1. Moreover, it remains unclear if peripheral administration of GLP-1R ligands mimics release of GLP-1 from the gut or, instead, activates GLP-1R normally involved in the responses to GLP-1 of central origin. Cells in the caudal NTS synthesize GLP-1, and GLP-1 receptors are found in the NTS as well as in various structures that receive projections from the NTS (22, 27). Several of these structures, including the lateral septum, amygdala, nucleus accumbens, preoptic area, PVN, supraoptic nucleus, subfornical organ, and area postrema, have a clear relevance to fluid intake and should be included in any preliminary list of structures that may be involved in the observed responses. Further studies are needed, however, to confirm or dismiss a specific role for any of these structures in GLP-1 receptor-mediated suppression of water intake.

The complicated regulation of water intake, and more generally, maintenance of body fluid homeostasis, requires consideration of several alternate explanations for the responses reported here. Specifically, it is important to consider the potential contribution of GLP-1R agonists, injected peripherally or centrally, on diuresis (21, 40), insulin secretion (18, 19, 35), blood pressure (1, 3, 8, 10, 15, 47), and visceral illness (17, 42, 44) before concluding that there is a more direct effect on water intake. Previous reports have shown that GLP-1 administration in rats causes diuresis as well as increased sodium excretion. It is unlikely, however, that these effects would contribute to the suppression of water intake observed following GLP-1R stimulation, as diuresis and increased sodium excretion would more likely generate compensatory increases in fluid and solute consumption. Whether or not GLP-1R agonists affect sodium intake remains a topic for further investigation. Although insulin affects water intake, the direction of insulin’s effect makes it unlikely that this explains GLP-1R-mediated hypodipsia because
administration of insulin increases, rather than decreases, water intake in the absence of food (39). Increased blood pressure inhibits water intake (33), but the time course of the pressor response to GLP-1 and the suppression of intake does not support changes in blood pressure as a more direct cause of the observed effects on water intake. Gardiner et al. (8) demonstrates that blood pressure returns to normal ~120 min after peripheral injection of exendin-4 (using a dose similar to that used in the present studies), but the suppression of water intake in the present study persisted for longer than 120 min. While we cannot completely discount a role for GLP-1R-mediated changes in blood pressure that could influence water intake, it is important to note that these blood pressure changes are more transient than the longer-lasting hypodipsic effects following GLP-1R activation, making it unlikely that the intake suppression was exclusively related to changes in blood pressure. It is also possible that the treatments used in the present study suppressed fluid intake by causing an illness-like response; however, we suggest caution before concluding that this was the primary reason for the decreased fluid intake. First, there is evidence that GLP-1-induced suppression of food intake and illness are separable. Microinjections of GLP-1R ligands into the PVN suppress food intake but do not produce a conditioned taste avoidance (CTA) and microinjections into the CeA produce a CTA without any suppression of food intake (17, 24). Second, Kinzig et al. (17) found that an injection of GLP-1 into the fourth ventricle was not sufficient to develop CTA, but this route of injection suppressed water intake in the present studies. Third, and perhaps most important, illness-inducing agents may not have the same effect on food intake as they do on fluid intake. Previous studies of water intake in response to LiCl poisoning found that water intake was either unaffected or increased by LiCl (5, 20, 38). These reports are of critical importance here because there is evidence that LiCl-induced illness may involve the GLP-1 system. Specifically, central administration of GLP-1R antagonists block LiCl-induced Fos, hypophagia, and pica (37, 41). We are not, however, arguing that illness-inducing effects of the drugs were absent in our studies. There is indeed evidence that GLP-1R agonists cause a sensation of visceral illness in humans (4) and a similar effect can be inferred from CTA studies in rats (17, 42, 44). However, based on the evidence presented above, we find this explanation insufficient to account uniformly for the suppressed water intake in the present studies. Thus,
it seems reasonable to conclude that any of these alternate explanations cannot entirely account for the hypodipsic effects following GLP-1R activation.

**Perspectives and Significance**

The present results provide an important extension of previous findings and help build support for a role for GLP-1 receptors in the regulation of water intake. Moreover, these results are likely generalizable because Gutzwiller et al. (9) found hypodipsia in humans after IV injections of GLP-1. Thus, the basic finding that GLP-1 receptor agonists decrease fluid intake could be especially important in clinical settings, especially with respect to geriatric patient populations that are already at risk for dehydration (6). Nevertheless, elucidating the precise role of GLP-1 receptors in fluid intake, determining the underlying neural substrates, and evaluating the possibility that any feeding responses are secondary to fluid intake effects requires further research. Collectively the data show that either peripheral or central administration of GLP-1 agonists suppress unstimulated water intake when food is unavailable.
Acknowledgements

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Disclosure

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References


Figure legends

**Figure 1.** Unstimulated water intake with food available after IP injection of vehicle (0.9% saline), GLP-1 (500 μg/kg), exendin-4 (3 μg/kg), or liraglutide (100 μg/kg). (a) Noncumulative 24 hr water intake collapsed into 4-hr bins. Both exendin-4 and liraglutide had a robust suppressive effect on water intake during the first 12 hours of testing (dark phase). Bars with different letters, and within the same bin, are significantly different (p<0.05). (b) Cumulative 24 hr water intake. Cumulative analysis illustrates that the initial hypodipsia after exendin-4 or liraglutide administration was not compensated for during the testing period. Asterisks indicate significant differences from vehicle (p<0.05). (c) Dark phase water intake. Exendin-4 or liraglutide suppressed water intake over the dark phase, with liraglutide administration causing the greatest suppression. Bars with different letters are significantly different (p<0.05). (d) Noncumulative water intake in 1-hr bins. Examination of shorter bins revealed an immediate, but transient, effect of GLP-1 on water intake. Asterisks indicate differences from controls within a given bin (p<0.05; data from the whole 24-h test were analyzed, but for clarity only the first 4 bins are shown in the figure). Data are shown as mean ± SEM.

**Figure 2.** Total 24 hr intake after IP injection of vehicle (0.9% saline), GLP-1 (500 μg/kg), exendin-4 (3 μg/kg), or liraglutide (100 μg/kg). Analysis of 24 hr food intake (a) showed a hypophagic response to exendin-4 or liraglutide that was markedly similar in direction and magnitude to the suppression of 24 hr water intake (b). The effect was normalized when the data were analyzed as the ratio of water intake to food intake (c). Bars with different letters within a given panel differed from each other significantly (p<0.05). Data are shown as mean ± SEM.

**Figure 3.** Unstimulated water intake without food available after IP injection of vehicle (0.9% saline), GLP-1 (500 μg/kg), exendin-4 (3 μg/kg), or liraglutide (100 μg/kg). (a) Noncumulative 24 hr water intake collapsed into 4-hr bins confirms that the hypodipsia produced by these drugs could not be explained by a primary suppression of food intake. Bars with different letters, and within the same bin,
are significantly different (p<0.05). (b) Cumulative 24 hr water intake illustrates that hypodipsia after GLP-1 or exendin-4 was compensated for by the end of the testing period. Asterisks indicate significant differences from vehicle (p<0.05). (c) Dark phase water intake. Of the three GLP-1R agonists, only liraglutide suppressed water intake over the entire dark phase. Bars with different letters are significantly different (p<0.05). (d) Noncumulative water intake in1-hr bins. Examination of shorter bins revealed an immediate, but transient, effect of GLP-1 on water intake, as was observed in Experiment 1. Asterisks indicate differences from controls within a given bin (p<0.05; data from the whole 24-h test were analyzed, but for clarity only the first 4 bins are shown in the figure). Data are shown as mean ± SEM.

**Figure 4.** Unstimulated intake without food available after LV injection of vehicle (0.9% saline), GLP-1 (10μg), exendin-4 (0.1μg), or liraglutide (2μg). (a) Noncumulative 24 hr water intake collapsed into 4-hr bins. Administration of these drugs centrally led to a similar pattern of hypodipsia as was seen after peripheral injections. Bars with different letters, and within the same bin, are significantly different at p<0.05. (b) Cumulative 24 hr water intake. Cumulative analysis illustrates that the initial hypodipsia after exendin-4 or liraglutide administration was not compensated for during the testing period. Asterisks indicate significant differences from vehicle (p<0.05). (c) Dark phase water intake. Exendin-4 or liraglutide administration suppressed water intake over the dark phase to a similar extent. Bars with different letters are significantly different (p<0.05). Data are shown as mean ± SEM. (d) Noncumulative water intake in1-hr bins. Examination of shorter bins revealed an effect of exendin-4, but not of any other GLP-1R agonists. Asterisks indicate differences from controls within a given bin (p<0.05; data from the whole 24-h test were analyzed, but for clarity only the first 4 bins are shown in the figure). Data are shown as mean ± SEM.

**Figure 5.** Unstimulated intake without food available after 4V injection of vehicle (0.9% saline), GLP-1 (10μg), exendin-4 (0.1μg), or liraglutide (2μg). (a) Noncumulative 24 hr water intake collapsed into 4-hr bins. Hindbrain administration of exendin-4 or liraglutide also led to suppression of water intake,
suggesting at least a partial contribution of the hindbrain in the observed hypodipsia. Bars with different letters, and within the same bin, are significantly different at p<0.05.  (b) Cumulative 24 hr water intake.  Cumulative 24 hr water intake suggests that GLP-1 also had a suppressive effect on water intake and that the initial hypodipsia after GLP-1, exendin-4, or liraglutide administration was not compensated for during the testing period. Asterisks indicate significant differences from vehicle (p<0.05).  (c) Dark phase water intake. GLP-1, exendin-4, or liraglutide administration suppressed water intake over the dark phase, with exendin-4 causing the greatest suppression. Bars with different letters are significantly different (p<0.05). Data are shown as mean ± SEM.  (d) Noncumulative water intake in 1-hr bins. Examination of shorter bins did not reveal any significant effects of GLP-1R agonists. Asterisks indicate differences from controls within a given bin (p<0.05; data from the whole 24-h test were analyzed, but for clarity only the first 4 bins are shown in the figure). Data are shown as mean ± SEM.
A

![Bar chart showing water intake (ml) over time (h) for different groups: Vehicle, GLP-1, Exendin-4, and Liraglutide.](image)

B

![Graph showing cumulative water intake (ml) over time (h) for different groups: Vehicle, GLP-1, Exendin-4, and Liraglutide.](image)

C

![Graph showing dark phase water intake (ml) for different groups: Vehicle, GLP-1, Exendin-4, and Liraglutide.](image)

D

![Graph showing water intake (ml) over time (h) for different groups: Vehicle, GLP-1, Exendin-4, and Liraglutide.](image)
McKay et al Figure 4

A

Water intake (ml)

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Time (h)

B

Cumulative water intake (ml)

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Water intake (ml)

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