Glucagon-Like Peptide-1 (7–36) Amide Administered into the Third Cerebroventricle Inhibits Water Intake in Rats (44320)

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Abstract. **Intracerebroventricular (icv) injection of glucagon-like peptide-1 (7–36) amide (GLP-1) has been shown to reduce food intake in rats. In these studies, we confirmed that injection of 10 μg of GLP-1 into the third cerebroventricle suppressed food intake. Moreover, we observed a reduction in water intake associated with the decreased food intake. We further examined whether GLP-1 injected icv in rats has a specific inhibitory effect on water intake. It was found that GLP-1 reduced water deprivation–induced drinking. Furthermore, the same dose of GLP-1 (10 μg) was sufficient to condition taste aversion. Finally, when 2 μg of GLP-1 were injected into the third ventricle, it only suppressed water deprivation–induced water intake and failed to influence spontaneous food and water intakes or induce conditioned taste aversion. These observations indicate that GLP-1 is a potent inhibitor of water intake in the rat and may play a role in the control of fluid homeostasis.** [P.S.E.B.M. 1998, Vol 219]

Glucagon-like peptide-1 (7–36) amide (GLP-1) is one of several post-transcriptional products of proglucagon. In the pancreatic α cells, proglucagon is processed to glucagon [proglucagon (33–61)], glicentin-related pancreatic peptide [proglucagon (1–30)], and c-terminal fragment [proglucagon (64–158)]. In the intestinal L cells, the predominant peptides present are glicentin [proglucagon (1–69)], oxyntomodulin [proglucagon (33–69)], and GLP-1 [proglucagon (78–108)]. In the brain, post-transcriptional processing of proglucagon is similar to that of intestinal L cells (1, 2), which also give rise to GLP-1.

In the periphery, GLP-1 has been shown to stimulate insulin secretion (1, 3), inhibit gastric acid secretion (4), and increase arterial blood pressure and heart rate (5). GLP-1 immunoreactivity and its binding sites are also found in the brain, with high concentrations in the hypothalamus and the nucleus of the solitary tract (NTS) (6–10). Studies conducted over the last several years have shown that intracerebroventricular (icv) injection of GLP-1 inhibits food intake and suggest that it is a physiological mediator of satiety (11–14). In earlier studies, water intake was either overlooked or measured at the same time that food intake was monitored. More recently, Tang-Christensen *et al.* (15) studied the role of GLP-1 in the control of water intake; however, it was not clear whether food was available when water intake was being measured. Because food intake and water intake are directly associated (2, 16, 17), the reduced food intake caused by central administration of GLP-1 could be related to the inhibitory effects of GLP-1 on water intake. Alternatively, GLP-1 may have an inhibitory effect on feeding behavior as well as drinking behavior. In addition, taste aversion was previously reported after GLP-1 was administered centrally (18). Therefore, it is also possible that GLP-1 injection produced a nonspecific inhibition of ingestive behavior by eliciting aversive sensory consequences.

The primary objectives of the following experiments were to determine the specific effect of GLP-1 on water

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intake. In addition, we investigated the potential aversive effect of third cerebroventricular GLP-1 injection using conditioned taste aversion (CTA) learning.

Materials and Methods

Animals and Housing. Adult male Sprague-Dawley rats (Harlan, Indianapolis, IN), initially weighing about 260–290 g at the time of intracranial surgery, were the subjects of all experiments. They were housed individually in suspended stainless steel cages in a temperature-controlled room on a 12hr:12hr light-dark cycle with the lights on at 0800 hr. They had free access to pelleted Purina chow (#5012) and tap water unless otherwise noted. A dim red light was used to provide illumination when some experimental procedures were performed in the dark phase. All experimental procedures involving the use of animals were conducted in accordance with NIH Guidelines and were reviewed and approved by the Animal Care and Use Committee for the University of Georgia.

Third Cerebroventricular Cannulation. All surgical procedures were performed using aseptic conditions. All rats were implanted with stainless steel guide cannulae into the third cerebroventricle under anesthesia with 1 ml/kg of a 3:2:1 v/v/v mixture of ketamine HCl (100 mg/ml Ketaset, Fort Dodge Laboratories, Inc., Fort Dodge, IA), acepromazine maleate (10 mg/ml PromAce, Fort Dodge Laboratories, Inc., Fort Dodge, IA), and xylazine (20 mg/ml Rompun, Miles Inc., Shawnee Mission, KS) or when necessary methoxyflurane (Metafane, Pittman-Moore, NJ). The dorsum of the head was shaved and vacuumed. The rat was placed in a stereotaxic instrument, and the skin was disinfected with chlorhexidine (Nolvasan, Fort Dodge, IA). The skin was incised to expose the skull over the forebrain. A burr hole was drilled in the skull over the bregma region. The cannula was introduced at an angle of 8° to the vertical plane. The stereotaxic coordinates used were: AP, −0.6 mm; ML, 0.9 mm with respect to bregma; and DV, −6.9 mm from the dura. The cannula was held in place with three stainless steel screws and cranioplastic cement (Plastics One, Roanoke, VA) attached to the skull. A 30-gauge stainless steel obturator was used to ensure that the cannula remained patent when the rat was not receiving an injection.

Drugs and Injections. Angiotensin II (ANG II, Sigma, St. Louis, MO) was dissolved in sterile artificial cerebrospinal fluid (aCSF) at a concentration of 20 ng/μl for the third ventricular injection. GLP-1 (Sigma) was dissolved in 0.9% physiological sterile saline (PSS) to a concentration of 2 μg/μl or 0.4 μg/μl for the third ventricular injection in Experiments 1, 2, and 3, and Experiments 4, 5, and 6, respectively. Control injections always consisted of an equivalent volume of the appropriate vehicle.

All the drugs injected into the third ventricle were given in a volume of 5 μl over 1 min. The rats were gently hand-held during injections. All rats were preconditioned to handling through daily measurement of body weight. The injections were carried out using an injector that was made from a 30-gauge stainless steel needle extending 1.5 mm beyond the tip of the guide cannula. The injector was connected by polyethylene tubing (PE 10) to a Gilmont micrometer syringe driven by a micrometric screw. Continuous infusion was verified by consistent movement of a small bubble (about 2 mm long) through the polyethylene tubing. The volume of injection fluid in the tubing was enough to prevent the air bubble from entering the third ventricle. Following injection, the injector was left in place for 1 min to allow equilibration of fluid in the injection line. The injector was then removed and replaced with the obturator.

Verification of Cannula Placement. At least 5 days after surgery, correct cannula placement was determined by an increased drinking response following ventricular injection of 5 μl ANG II (100 ng) compared to an injection of 5 μl aCSF. If a rat did not increase drinking in response to ANG II, it was eliminated from the study. At the end of each experiment, cannula placement was verified again by an increased drinking response to ANG II. After completing all experiments, the rats were euthanized with sodium pentobarbital and perfused with 10% formalin transcardially, followed by injection of 5 μl 0.14% methylene blue solution into the ventricle. Cannula placement was verified histologically. Only data from the rats with a positive drinking response to ANG II, presence of dye in the ventricular system, and correct cannula placement were used in the statistical analysis.

Experiment 1. Food and Water Intake Test. Twenty-eight naive rats were surgically prepared with guide cannulae into the third ventricle. At least 5 days after surgery, the ANG II screening test was performed. Eighteen rats responded by drinking at least 1.5 ml or more water in the 30 min following ANG II injection than following aCSF injection. These rats were the subjects of this experiment. They were randomized into two groups, GLP-1 (n = 9) and PSS (n = 9). On the experimental day, the food cups and water bottles were replaced with weighed pelleted Purina chow and calibrated drinking tubes 30 min before the onset of the dark phase. After this 30-min acclimation period, 10 μg of GLP-1 or 5 μl of PSS was injected into the third ventricle. Food and water intakes during the dark cycle in nonfood and water-deprived rats were measured at 0.5, 1, 2, 3, 16, and 24 hr after treatment, and comparisons were made between GLP-1 and PSS treatments.

Experiment 2. Water Deprivation-Induced Drinking Test. Eighteen rats were divided into two treatment groups, i.e., GLP-1 (n = 9) and PSS (n = 9). Each group consisted of half the animals randomly selected from the GLP-1 treated group and the other half from the PSS-treated group in Experiment 1. Drinking was elicited by 9 hr water deprivation. Water bottles were removed from the cages at 0200 hr. The rats had free access to food during the 9-hr period (i.e., from 0200 hr to 1100 hr). At 1100 hr, food cups were removed from the cages and 10 μg of GLP-1 or 5 μl of PSS was injected into the third ventricle. Immediately after injection, the rats were given calibrated drinking

tubes filled with water. The volume of water consumed was monitored at 0.5, 1, 2, 3, 4, and 5 hr. Four days after this experiment, an ANG II screening test was performed. Three rats did not drink in response to ANG II infusion. Data from these rats were eliminated prior to statistical analysis, thus, group sizes were GLP-1 treated group, $n = 8$; and PSS-treated group, $n = 7$.

Experiment 3. CTA Test. Twenty-eight naive rats were implanted with the third cerebroventricular guide cannula. At least 1 week after the surgery, 17 rats had a positive drinking response in an ANG II screening test. They were randomly divided into two treatment groups: 10 μg of GLP-1 injected ($n = 9$), and 5 μl of PSS injected ($n = 8$). All behavioral testing was performed during the light phase of the light-dark cycle. On experimental Days 1 to 5, food cups and water bottles were removed from the cages for 30 min. During this 30-min period, rats were trained to drink a water-diluted, sweetened condensed milk solution from calibrated drinking tubes. The milk solution was made from 1 part sweetened condensed milk (Meadow Gold, Meadow Gold Dairies, Inc., Columbia, OH) diluted with 2 parts distilled water. Milk intake was measured at 15 and 30 min. After the training, food and water were returned to the cages. On Day 6, the same procedures as on Days 1 to 5 were repeated except that after the milk was removed from the cages, the rats were injected with 5 μl PSS into the third ventricle to condition them to the injection procedure. Three hr later, weighed food cups and calibrated drinking tubes filled with water were returned to the cages. On Day 7 (conditioning trial), after water drinking tubes and weighed food cups were removed, all the rats were given the same diluted sweetened condensed milk but with a novel almond flavor (the conditioned stimulus, CS) for 30 min, and their intake was recorded at 15 and 30 min. At the end of the 30-min test period, rats were injected with GLP-1 or PSS (the unconditioned stimulus, US). Three hr later, water drinking tubes and food cups were returned to the cages. Seventy-two hr later (Day 10, retention trial), all rats were presented with the same almond-flavored sweetened milk, and their intake, without water and regular chow available, was recorded for 30 min. Food and water were then returned to the cages. If the rats consumed as much or more milk than they consumed on test Day 7, they were considered not to develop an aversion. From Days 5 to 10, daily regular chow and water intakes were monitored to ensure that GLP-1 had no effect on regular chow and water intakes.

Experiment 4. Water Deprivation–Induced Drinking Test. Thirty days after Experiment 3, the ANG II test was performed on the 28 rats that were surgically prepared for Experiment 3. Twenty rats had a positive drinking response. They were used for the water deprivation–induced water intake test as described in Experiment 2 except that 11 rats were treated with PSS, and 9 rats were injected with 2 μg of GLP-1.

Experiment 5. Food and Water Intake Test. Forty days after Experiment 3, 20 rats that still had a positive drinking response to ANG II were used for the same food and water intake test as described in Experiment 1 except that 10 rats were treated with PSS, and 10 rats were injected with 2 μg of GLP-1.

Experiment 6. CTA Test. Twelve naïve cerebroventricularly cannulated rats were the subjects of this CTA test. The experimental procedures were the same as described in Experiment 3 except that rats were treated with icv 2 μg of GLP-1 ($n = 6$) or 5 μl of PSS ($n = 6$).

Data Analysis. Repeated measures analysis of variance (ANOVA) was used for statistical analysis of data in Experiments 1, 3, 5, and 6. If an overall significance was found in the two-way ANOVA, a Newman-Keuls *post-hoc* analysis was used to compare differences between groups at each time point. The data in Experiments 2 and 4 were analyzed by Fisher's exact test for equality of proportions. A confidence level of $P < 0.05$ was regarded as significant.

Results

Food and Water Intake Tests. There was a significant difference in food intake and water intake between the 10-μg GLP-1–injected group and the PSS-injected group as revealed by a two-way ANOVA, $F(16, 80) = 13.43$, $P < 0.01$ and $F(16, 80) = 5.57$, $P < 0.05$, respectively. Cumulative food intake of the GLP-1–treated rats, compared to that of the PSS-treated rats, was significantly suppressed by 57.3%, 62.6%, 19.3%, and 19.1% at 2, 3, 16, and 24 hr after treatment, respectively (Fig. 1A, $P < 0.02$). Cumulative water intake was also markedly decreased by 92.6%, 91.6%, and 33.7% after GLP-1 treatment, as compared to PSS treatment at 2, 3, and 16 hr, respectively (Fig. 1B, $P < 0.05$). Thus, at 2, 3, and 16 hr, the percentage of water intake suppression was greater than that of food intake suppression. As indicated by analysis of the fractional intake during the different time periods, GLP-1 produced an inhibition in food intake during the intervals of 0.5 hr–1 hr, 1 hr–2 hr, and 2 hr–3 hr (Fig. 1C, $P < 0.01$). There was no compensatory feeding for up to 24 hr. During the intervals of 1 hr–2 hr and 2 hr–3 hr water intake was significantly reduced by GLP-1 ($P < 0.05$), whereas during 16 hr–24 hr there was a compensatory increase in water intake in the GLP-1 injected group (Fig. 1D, $P < 0.01$). Thus, analysis of the fractional intake during the different time periods of the experiment indicated that the inhibitory effects of GLP-1 on food and water intakes were mainly present in the first 3 hr of the dark phase following injection.

During the entire 24-hr test period, there was no significant difference in food intake and water intake between the 2 μg GLP-1–injected group and the PSS-injected group as revealed by a two-way ANOVA, $F(17, 85) = 1.34$, $P = 0.26$ and $F(17, 85) = 0.40$, $P > 0.05$, respectively (Fig. 2).

Water Deprivation-Induced Drinking Tests. The effect of GLP-1 on water intake and the independence of this effect from the presence of food was demonstrated using the rats that were water deprived (food *ad libitum*) for 9 hr. After 9 hr of water deprivation, 5 μl of GLP-1 (dose 10

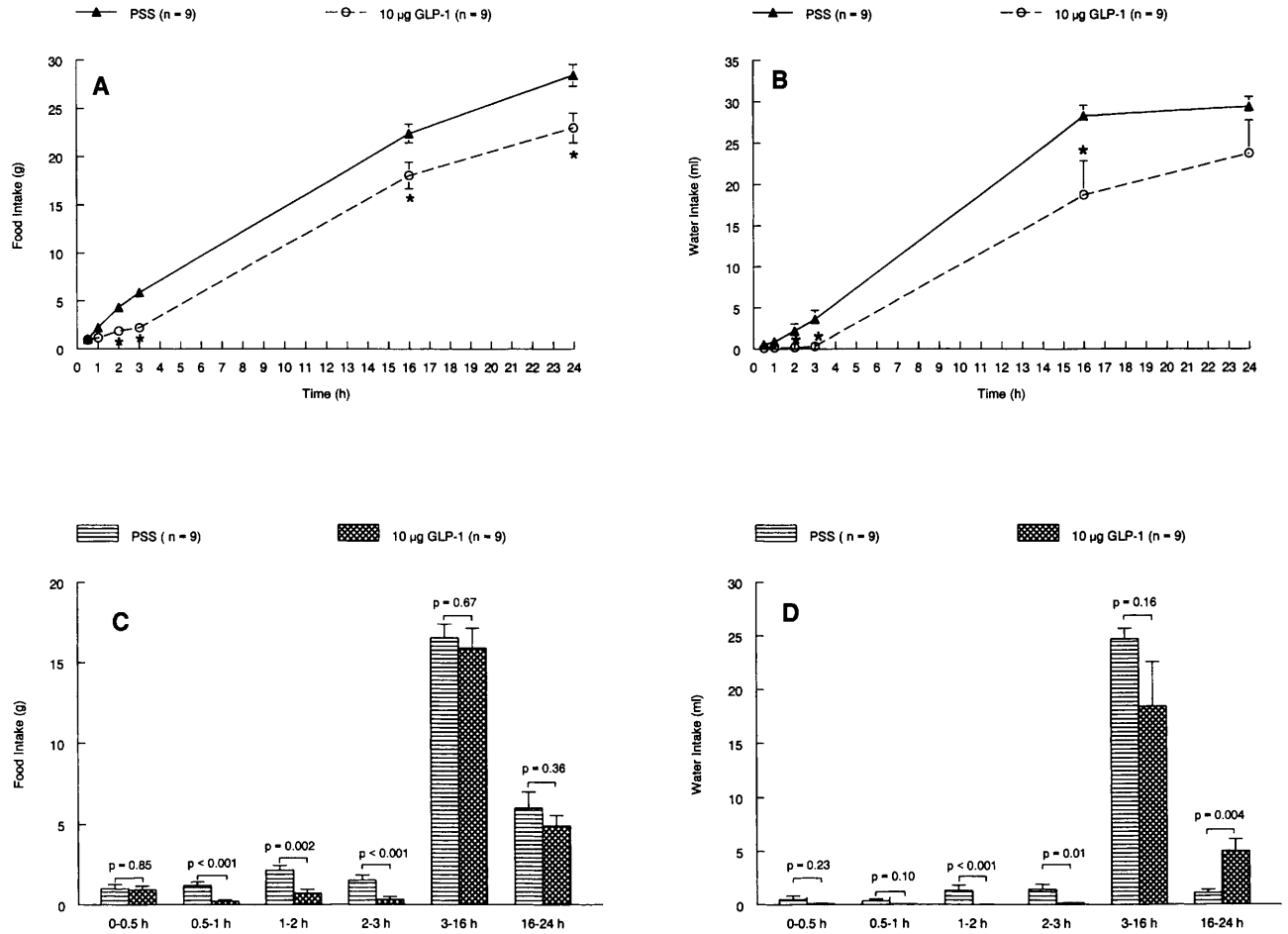


Figure 1. Suppression of cumulative food intake (A) and water intake (B) by third cerebroventricular injection of 10 μg GLP-1. *Significantly less than physiological sterile saline-treated group ($P < 0.05$). Effect of 10 μg GLP-1 administration on food intake and water intake during different time intervals of feeding and drinking test are illustrated by (C) and (D), respectively. Data are expressed as mean food intake (g) or water intake (ml) ± SEM.

μg) or PSS was injected into the third ventricle immediately prior to placing the drinking tubes to the cages. During the subsequent 5 hr, without food present in the cages, none of the GLP-1–treated rats drank water whereas the control rats drank 4.4 ± 1.2 ml (Fig. 3, $P < 0.05$).

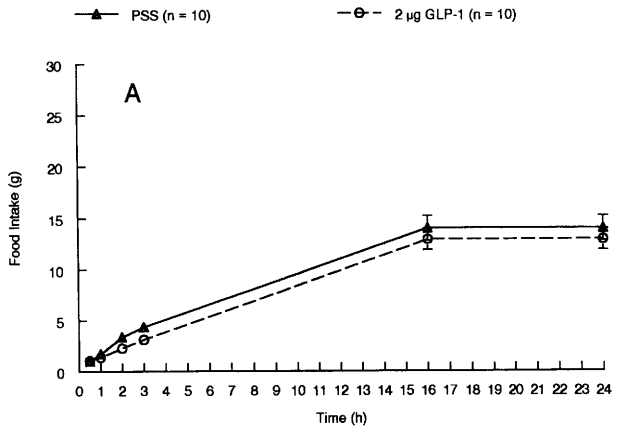
During the 5-hr drinking test after 9 hr of water deprivation, water intake for the 5 μl PSS and 2 μg GLP-1–injected groups were 4.6 ± 1.0 ml and 0.3 ± 1.1 ml, respectively (Fig. 4, $P < 0.001$).

Conditioned Taste Aversion Test. In Experiment 3, data from one rat that drank less than 1 ml of almond-flavored milk during the conditioning trial were eliminated. Thus, statistical analyses were performed on the following groups: icv 5 μl PSS injected, $n = 8$; and icv 10 μg GLP-1 injected, $n = 8$.

During the retention trial, the consumption of the almond-flavored sweetened milk, CS, was significantly different between the 10 μg GLP-1–injected and the PSS-injected groups as revealed by overall ANOVA, $F(14, 14) = 30.07$, $P < 0.001$. It was reduced 57% in the 10 μg GLP-1–injected rats, indicating a CTA to almond-flavored sweetened milk (Fig. 5). However, PSS-injected rats drank significantly more milk during the retention trial than during the conditioning trial ($P < 0.01$), indicating that they failed to form a CTA. During the conditioning trial, the two groups of rats had comparable milk intakes (PSS group, 5.3 ± 0.7 ml; GLP-1 group, 4.2 ± 0.8 ml; $P > 0.05$). Thus, it seems unlikely that the decreased intake by GLP-1–treated rats in the retention trial is due to overconsumption during the conditioning trial that induces a distaste for the previously favorite food or because of the difference in endogenous, food ingestion–elicited, circulating satiety factor(s) between the two groups of rats.

From experimental Days 5 to 10, daily regular chow intake, water intake, and body weight of the two groups were similar ($P > 0.05$, $P > 0.05$, and $P > 0.05$, respectively). These observations indicate that the two treatments did not have an effect on regular chow and water intake and body weight as measured from 24 hr–72 hr after injection. Therefore, intake during the retention trial was not the result of differences in daily food and water intake.

In Experiment 6, the consumption of the almond-flavored sweetened milk was not significantly different between the 2 μg GLP-1–injected and PSS-injected groups



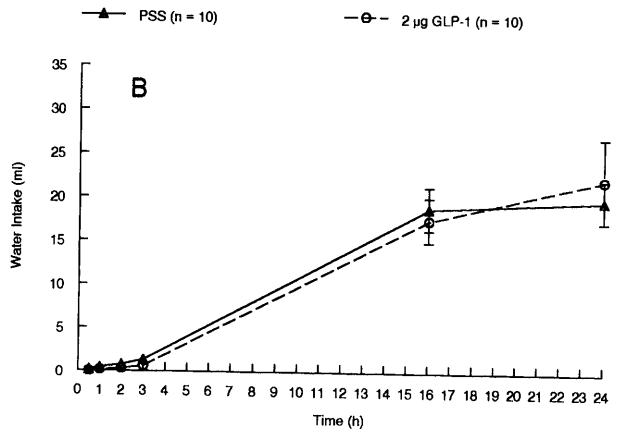


Figure 2. Cumulative food intake (A) and water intake (B) after third cerebroventricular injection of 2 µg GLP-1 or 5 µl PSS. Data are expressed as mean food intake (g) or water intake (ml) ± SEM.

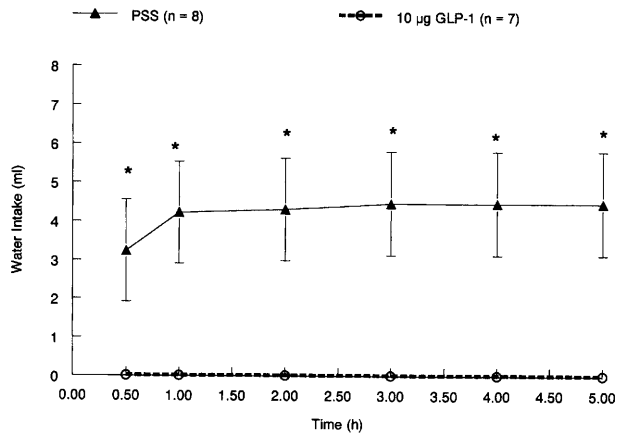


Figure 3. Cumulative 5-hr water intake after 9 hr of water deprivation. A complete inhibition of water intake was observed in the GLP-1 (dose 10 µg)–injected group. *The cumulative water intake by the PSS-injected group was significantly greater at 0.5, 1, 2, 3, 4, and 5hr after injection as compared to the 10 µg GLP-1–injected group ($P < 0.05$).

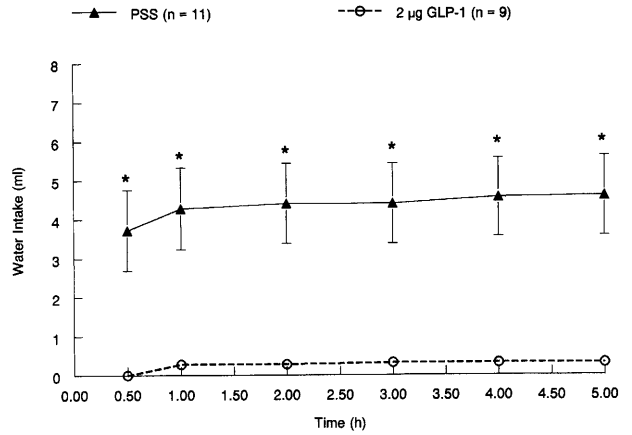


Figure 4. Cumulative 5-hr water intake after 9 hr of water deprivation. A complete inhibition of water intake was observed in the GLP-1 (dose 2 µg)–injected group. *The cumulative water intake by the PSS-injected group was significantly greater at 0.5, 1, 2, 3, 4, and 5hr after injection as compared to the 2 µg GLP-1–injected group ($P < 0.05$).

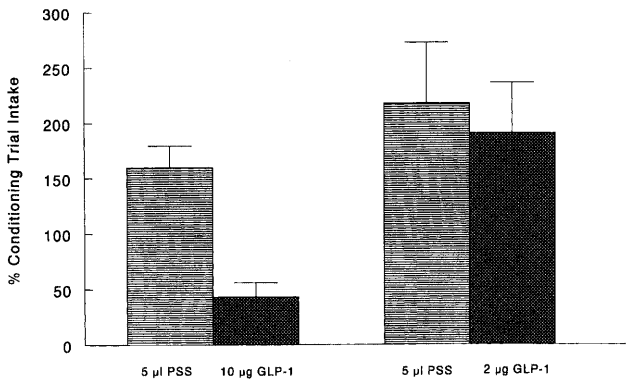


Figure 5. Novel diet (almond-flavored sweetened milk, conditioned stimulus) intake by rats with PSS injection or GLP-1 injection (unconditioned stimulus) during the retention trial of CTA expressed as a percentage of conditioning trial intake. Data are expressed as mean percentage ± SEM.

during either the conditioning or the retention trials ($P > 0.05$). Both GLP-1–treated and PSS-treated rats tended to consume more milk during the retention trial than during the conditioning trial, indicating that they failed to develop a CTA (Fig. 5).

Discussion

The inhibition of food intake by administration of substances is often accompanied by a concomitant suppression of water intake (19). It could be that animals do not drink simply because they do not eat. Previous studies have shown an anorexic effect of GLP-1 but have not taken into consideration water intake (11–14). When the present studies were performed, to our knowledge, only one study had observed a concomitant decrement in food intake and water intake following icv administration of GLP-1 (13). In this case, however, it was not clear whether suppression of drinking was a direct effect of GLP-1 on drinking *per se* or a secondary effect due to a decrease in food intake (prandial

drinking). We found that a dose of GLP-1 effective in inhibiting food intake, induced not only a concomitant reduction in water intake in Experiment 1, but also adipsia when tested in the absence of food in Experiment 2. Our results showed that the inhibitory effect of GLP-1 on drinking is not dependent on food intake.

If a rat is injected with an agent (unconditioned stimulus) that results in malaise or illness, immediately after the rat drinks a novel flavored fluid (conditioned stimulus), the rat will acquire an aversion to the taste of the fluid. This is commonly referred to as conditioned taste aversion. Our data demonstrated that icv injection of GLP-1 suppressed spontaneous food and water intakes at the same time and completely abolished water intake to a water deprivation stimulus. It can be argued that the inhibition of ingestive behavior following such GLP-1 injection does not reflect a specific inhibition of ingestion but rather results from a general malaise that interferes with the behavior of the animal. Thus, in our studies, we made an attempt to examine the possibility of malaise or nausea being responsible for the reductions in intake. We found that the same dose of GLP-1, given intracerebroventricularly, that produced reductions in eating and drinking appeared to be an effective unconditioned stimulus when tested in a CTA paradigm in the present study. Our results are consistent with those reported by Thiele *et al.* (18) that centrally administered 10 μg GLP-1 elicited CTA. Two other measures of CTA learning (latency to reject oral saccharin and a two-bottle, saccharin-versus-water preference test) were used in their study. Therefore, the possibility that GLP-1 injection produced a nonspecific inhibition of ingestive behavior by producing an aversive sensory consequence cannot be excluded. However, the production of a CTA, using a single dose of GLP-1 as the unconditioned stimulus, is not a sufficient test to conclude that GLP-1 is not a satiety agent, and it does not have a specific inhibitory effect on drinking. Tang-Christensen *et al.* (15) reported results on GLP-1 at the dose of 1 μg administered into the lateral ventricle in their CTA studies. This dose of GLP-1 failed to elicit CTA and did not have significant effect on drinking under two models of thirst induction (ANG II administration and a water restriction schedule). Higher doses of GLP-1 (i.e., 2- and 10-μg doses) were required for inhibiting ANG II-induced drinking and a water restriction schedule-induced drinking, respectively. The results from the present study indicated that 2 μg of GLP-1 did not result in CTA or decreases in spontaneous food and water intakes after the onset of dark phase. Nonetheless, this dose inhibited water deprivation–induced drinking. All these observations suggested that low doses of GLP-1 that are not aversive suppress water intake. Furthermore, the significant effect of the specific GLP-1 antagonist, exendine (9–39) amide, on ingestive behavior also demonstrated a physiological role for GLP-1 in regulating feeding and drinking (14, 15).

In summary, 10 μg of GLP-1 injected into the third cerebroventricle resulted in suppression of feeding as well as drinking behavior. This dose also produced a conditioned taste aversion. When the dose of GLP-1 was lowered to 2 μg, it selectively inhibited water intake without any effect on food intake. These findings suggest that GLP-1 plays a specific inhibitory role in the control of water intake. The specific mechanisms and sites through which it induces this effect remain to be elucidated. The hypothalamus, the subfornical organ, the area postrema, and the NTS are the brain regions with highest densities of GLP-1 binding sites (7, 14). These regions play an important role in the control of food and fluid intake (see Refs. 20–22 for review). Further studies targeting these areas will help us understand the role of GLP-1 in the regulation of energy and fluid homeostasis.

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