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The effect of ghrelin on water intake during dipsogenic conditions

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Abstract

Ghrelin has been studied extensively in the context of food intake and energy homeostasis, but less is known about its role in other ingestive behaviors. The present studies investigated the effects of this orexigenic peptide on both food and water intake during dipsogenic conditions. Specifically, animals were exposed to one of five dipsogenic stimuli: (1) 24-hr water deprivation, (2) replacement of drinking water with 2.5% NaCl, (3) peripheral administration of hypertonic saline, (4) ICV injection of angiotensin II (AngII), or (5) the combination of peripheral hypertonic saline and central AngII. Animals then were given an ICV injection of ghrelin (0.5 μ g) or vehicle, and subsequent food and water intakes were measured. Ghrelin reliably increased food intake under each stimulus condition. Ghrelin also affected water intake, but with less consistency across the conditions. Specifically, ghrelin attenuated water intake stimulated by acute injection of AngII or hypertonic saline, but failed to affect drinking in the other three stimulus conditions. Investigation of the temporal pattern of food and water intakes in three of these dipsogenic conditions failed to support a role of different intake patterns in the observed differences in water intake by ghrelin-treated rats. Although the effect of ghrelin on water intake was not present in every dipsogenic condition, these data provide evidence that the actions of ghrelin are not limited to food intake, but can also include alterations in water intake.

Keywords

ghrelin; drinking; water intake; food intake; angiotensin; fluid balance; dipsogen; hypertonic saline; water deprivation

Introduction

Ghrelin is a peptide that has been studied extensively in relation to food intake. It is produced primarily in the gastrointestinal tract [1,2] and exogenous administration of ghrelin to the periphery or brain increases food intake in laboratory animals [3,4] and humans [5]. Recent reports using ghrelin receptor antagonists provide important support for a role of endogenous ghrelin in food intake [6].

The sites of action that give rise to the hyperphagic effect of ghrelin have been well studied and include areas in the hypothalamus and caudal brainstem often associated with feeding behavior [4,7–16]. More recently, however, it has become apparent that additional brain areas respond to ghrelin, including those more traditionally associated with the regulation of body

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fluid homeostasis. For example, the subfornical organ (SFO) has been shown to contain ghrelin-responsive neurons *in vitro* [17]. Despite the progress that has been made in identifying the effects of ghrelin on feeding and the purported ability of ghrelin to act at brain structures involved in fluid balance, the effect of ghrelin on drinking has received relatively little attention [18,19].

In addition to the inclusion of the SFO in the list of targets of ghrelin, several pieces of evidence point to an interesting role for ghrelin in water intake. First, although ghrelin reliably increases food intake, the expected attendant prandial drinking seems to occur far less consistently [20]. Moreover, a recent report described ghrelin-induced inhibition of water intake in water-deprived rats [21], but tested only one stimulus for drinking. Additionally, obestatin, a peptide related to ghrelin, has been shown to have anti-dipsogenic effects [22,23]. To further explore the relationship between ghrelin and water intake in the laboratory rat, we examined the effect of a hyperphagic dose of ghrelin when water intake was stimulated by various approaches. Data from these experiments support the role of ghrelin as an anti-dipsogen under some, but not all circumstances.

Method

Animals

Eighty-seven adult, male Sprague Dawley rats (Charles River Laboratories Inc., Wilmington, MA) were used for the experiments. Animals were housed individually in hanging wire cages in a temperature-controlled environment under a 12-hr light/12-hr dark cycle (lights on 0600–1800 hr). Food and water were available *ad libitum* except where noted. Animals were allowed to acclimate to their cages for a week prior to surgery. A subset of rats were used in multiple experiments, but never more than 2 experiments, and care was taken to ensure proper balance in the group assignments such that previous experience did not account for the observed results. Moreover, animals subjected to conditions with a greater potential for long-term consequences (e.g., 24 hr deprivation and replacing drinking water with NaCl) were not used in any subsequent experiments. Indeed, repeated use of subjects was minimal with only 13 animals used more than once. All experimental protocols were approved by the Institutional Care and Use Committee of the State University of New York at Buffalo and were consistent with the *National Institutes of Health Guide for Care and Use of Laboratory Animals*.

Surgery

All experimental animals were implanted with chronic indwelling cannulae aimed at the lateral ventricle. Rats were anesthetized (70 mg/kg ketamine and 5 mg/kg xylazine) and placed in a stereotaxic apparatus. A 26 ga guide cannula (Plastics One Inc., Roanoke, VA) was implanted (coordinates: 0.9 mm posterior to bregma, 1.4 mm lateral to midline, and 1.8 mm ventral to dura) and affixed with bone screws and dental cement. Animals were allowed to recover for one week before proper cannula placement was verified by injection of 10 ng AngII (Bachem Bioscience Inc., King of Prussia, PA) in 1 μ L Tris-buffered saline (TBS) using a 33 ga injection cannula fabricated to extend beyond the guide cannula into the lateral ventricle. Animals were included in the experiments if they drank at least 5 mL of water in the 30 min post-injection. Animals were used for experiments beginning approximately one week after verification.

Drug injections and intake measures

All experiments were initiated during the early portion of the lights-on period. Animals received an ICV injection of drug or vehicle, after which graduated water bottles were placed on the cages, food hoppers containing a pre-weighed amount of food were provided, and plastic transparencies were placed underneath the cages to catch any food spillage. Water intake was measured at 30, 60, and 240 min after injection and food intake was measured at 240 min. After

final measurements were recorded, the transparency was removed and the crumbs were weighed and accounted for in the measurement of total food consumed during the 4-hr period.

Experiment 1: Effect of ghrelin on intake after 24-hr water deprivation

Animals were deprived of water, with food available *ad libitum* for 24 hr. Animals then received an ICV injection of 0.5 µg rat ghrelin (Bachem Bioscience Inc., King of Prussia, PA) in 1 µL artificial CSF (aCSF; Harvard Apparatus, Holliston, MA) or vehicle (1 µL aCSF; *n*=8 rats per group). This is a moderate dose of ghrelin that induces hyperphagia when administered ICV [8,9,12] and previously was shown to reduce water intake in rats [21]. Immediately after ghrelin injection, access to water was restored. Food and water intakes were measured as described above.

Experiment 2: Effect of ghrelin on intake by animals maintained on hypertonic saline for 24 hr

Following a modified experimental paradigm established by Watts and colleagues (for review see [24]), drinking water was replaced with 2.5% NaCl for 24 hr. Food was available *ad libitum* during this time. Animals then received an ICV injection of 0.5 µg rat ghrelin or vehicle (1 µL aCSF; *n*=6–7 rats per group). Immediately after injection, access to water was restored. Food and water intakes were measured as described above.

Experiment 3: Effect of ghrelin on intake after peripheral administration of hypertonic saline

Animals received a subcutaneous injection of 0.1 mL Lidocaine (2%) followed by a second SC injection of 2 mL hypertonic NaCl (2 M). Food and water were removed for 30 min. Immediately before food and water were returned, animals received an ICV injection of either 0.5 µg rat ghrelin or vehicle (1 µL aCSF; *n*=5 rats per group). Food and water intakes were measured as described above.

Experiment 4: Effect of ghrelin on intake after central administration of AngII

Animals received one ICV injection containing 0.5 µg ghrelin, 10 ng AngII, both, or vehicles only (1 µL aCSF for ghrelin; 1 µL TBS for AngII; *n*=7–8 rats per group). Food and water intakes were measured as described above.

Experiment 5: Effect of ghrelin on intake after peripheral administration of hypertonic saline and central administration of AngII

This experiment combined portions of experiments 3 and 4. As in experiment 3, all animals received a subcutaneous injection of 0.1 mL Lidocaine (2%) followed by a second SC injection of 2 mL hypertonic NaCl (2 M). Food and water were removed for 30 min. Immediately before food and water were returned, animals received an ICV injection containing 10 ng AngII in 1 µL TBS and either 0.5 µg ghrelin or vehicle (1 µL aCSF; *n*=7 rats per group). Food and water intakes were measured as described above.

Experiment 6: Time course of the effects of ghrelin on food and water intake after exposure to dipsogenic stimuli

To address the possibility that patterns of food or water intake differed among dipsogenic conditions in a manner that could explain the differences in the adipsic potency of ghrelin, we examined the time course of food and water intake in animals injected with ghrelin alone (0.5 µg in 1 µL aCSF) or under one of three dipsogenic conditions: 24-hr water deprivation, peripheral administration of hypertonic saline, or central administration of AngII (*n*=4–5 per group). The generation of the dipsogenic condition was identical to the methods and doses

described above in Experiments 1, 3, and 4. Food and water intakes were measured every 30 min after the injection of ghrelin for a total of 4 hr.

Statistical Analysis

Data for water intake were analyzed using SPSS for Windows (version 14.0). For each experiment, a mixed design ANOVA was used to test for between-subjects effects while accounting for the repeated intake measures for each animal. Accordingly, all main effects reported, except time, are between subjects. For experiments 4 and 6, Student-Newman-Keuls posthoc tests were completed using Sigma Stat (version 3.1) when separate two-way ANOVAs on data from individual times revealed a statistically significant interaction effect. Food intake data were analyzed by one- or two-way ANOVA using SPSS.

Results

Experiment 1: Effect of ghrelin on intake after 24-hr water deprivation

To evaluate the effect of ghrelin on water intake stimulated by water deprivation, we water deprived rats for 24 hr and measured water and food intakes after injection of ghrelin or vehicle and restoration of access to water. As shown in Figure 1A, ghrelin reliably increased food intake ($F_{1,14}=5.572$; $p=0.033$). In spite of the increases in food intake stimulated by ghrelin, we observed no statistically significant difference in water intake between groups (Figure 1B; $F_{1,14}=2.352$; $p=0.147$).

Experiment 2: Effect of ghrelin on intake after maintaining animals on hypertonic saline for 24 hr

To further evaluate the effect of ghrelin on water intake, we replaced drinking water with 2.5% NaCl for 24 hr. This manipulation is a potent stimulus for drinking [24]. Immediately before reinstating access to water, animals received an ICV injection of either vehicle or ghrelin. As expected, ghrelin increased food intake (Figure 2A; $F_{1,11}=34.774$; $p<0.001$), but vehicle- and ghrelin-treated animals drank similar amounts of water ($F_{1,11}=0.238$; $p=0.635$; Figure 2B).

Experiment 3: Effect of ghrelin on intake after peripheral administration of hypertonic saline

To determine the effect of ghrelin on a relatively acute stimulus for drinking, we examined the effects of ghrelin on water intake induced by injection of hypertonic saline. Thirty min after a single SC injection of 2M NaCl, ICV injection of ghrelin increased food intake (Figure 3A), but water intake by ghrelin-treated animals was markedly less than observed for animals injected with aCSF (Figure 3B). Statistical analysis of these data confirmed the effect of ghrelin on intake of food ($F_{1,8}=14.126$; $p<0.01$) and water ($F_{1,8}=12.938$; $p<0.01$).

Experiment 4: Effect of ghrelin on intake after central administration of AngII

The stimulus for water intake used in Experiment 3 was more acute than those used in Experiments 1 and 2. In addition to differences in the time course of the stimuli, the conditions in Experiments 1 and 2 offer less control over the selective perturbations of intracellular or extracellular fluid compartments, whereas the injection of hypertonic saline used in Experiment 3 produces only intracellular dehydration. To determine if ghrelin also affected drinking stimulated by a hormonal signal related to hypovolemic thirst, we measured the effect of ghrelin on water intake stimulated by AngII. Ghrelin reliably increased food intake in animals with and without AngII (Figure 4A; $F_{1,25}=25.526$; $p<0.001$), but injection of AngII alone had no effect on food intake ($F_{1,25}=0.524$; $p=0.476$). In contrast, ghrelin alone had no effect on water intake, but it reduced AngII-induced water intake (Figure 4B). Statistical analysis of these data confirmed that animals treated with AngII drank more water than vehicle-treated animals ($F_{1,25}=88.159$; $p<0.001$). Animals treated with ghrelin and AngII drank less water than animals

given only AngII ($F_{1,25}=17.663$; $p<0.001$), although these animals drank more than animals given only vehicle or ghrelin.

Experiment 5: Effect of ghrelin on intake after peripheral administration of hypertonic saline and central administration of AngII

Experiments 3 and 4 demonstrated that acute dipsetic stimuli produce drinking that is attenuated by concurrent administration of ghrelin. These experiments examined, respectively, two different types of stimuli: intracellular dehydration and a hormonal signal relevant to hypovolemia. As such, Experiment 5 was designed to test for anti-dipsogenic effects of ghrelin when water intake was produced by acute stimuli related to intracellular and extracellular thirst. We measured the effect of ghrelin on food and water intake of animals treated with a subcutaneous injection of hypertonic saline combined with ICV administration of AngII. Animals treated with ghrelin showed reliable increases in food intake (Figure 5A; $F_{1,12}=7.705$; $p<0.05$), but ghrelin had no observed effect on water intake (Figure 5B; $F_{1,12}=1.807$; $p=0.204$).

Experiment 6: Time course of the effects of ghrelin on food and water intake after exposure to dipsogenic stimuli

Experiments 1–5 demonstrated that ghrelin acts as an anti-dipsogen under some, but not all, dipsogenic conditions. In order to determine whether or not the differential effects of ghrelin on intake were due to differences in the temporal pattern of water or food intake, we examined three of the dipsogenic conditions in further detail: peripheral administration of hypertonic saline, central administration of AngII, and 24-hr water deprivation. The experiments described above showed that ghrelin was able to attenuate water intake under the first two conditions, but we found no effect of ghrelin during the third. As such, we were testing for common patterns of intake in the first two conditions that differ from the third. In this experiment, animals received 0.5 μg of ghrelin, either by itself or in conjunction with one of the three dipsogenic stimuli. Food and water intakes were measured every 30 min for a total of 4 hr.

Analysis of cumulative (data not shown) and non-cumulative food intake revealed a main effect of treatment (Figure 6A; $F_{1,14}=6.508$; $p<0.01$), without an effect of time ($F_{1,14}=3.658$; $p=0.076$) or an interaction ($F_{3,14}=0.585$; $p=0.635$). Post-hoc tests (Student-Newman-Keuls) revealed that food intake by animals deprived of water for 24 hr differed from all other groups, but differences between other groups were not detected.

Analysis of cumulative (data not shown) and non-cumulative water intake suggested that ghrelin attenuated drinking in AngII- and hypertonic saline-treated animals, but not in animals that were water-deprived for 24 hr (Figure 6B). Specifically, animals in the AngII and hypertonic saline conditions drank less water than animals deprived of water for 24 hr, consistent with reduced drinking in ghrelin-treated animals observed under these conditions in the experiments described above. Statistical analysis of these data using a mixed-design two-way ANOVA confirmed significant main effects of time ($F_{1,14}=87.470$; $p<0.001$) and treatment ($F_{1,14}=15.821$; $p<0.001$) and a significant interaction ($F_{3,14}=13.208$; $p<0.001$). Subsequent analyses on data from individual times using separate one-way ANOVAs revealed between-subjects differences in water at 30, 90, and 120 min (all F -values >3.087 , p -values <0.013). Student-Newman-Keuls post-hoc tests on the data from the first 30 min indicated that animals in the 24-hr deprivation condition drank more than any other group and that the ghrelin-treated animals drank less than all other groups. Analysis of data collected 90 min after injection of ghrelin showed that water-deprived animals drank more than animals in all other groups. Similarly, at 120 min, water-deprived animals drank more than animals in the ghrelin-alone and AngII+ghrelin groups, but no other comparisons were significant.

To further evaluate the pattern of water and food intake under the conditions tested, we calculated the percent of water and food intake occurring at each measured interval. As shown in Figure 7, the data fail to reveal any systematic differences in patterns of food or water intake that might account for the different effects of ghrelin on water intake observed in the different dipsogenic conditions.

Discussion

Ingestive behavior has become a well-studied topic in recent years as several potentially critical health issues have entered the public eye. Conditions such as obesity and hypertension are often comorbid and involve both food intake and body fluid homeostasis, highlighting the need to understand the interplay between the regulation of feeding and drinking behaviors. The present experiments focused on the interaction between ghrelin, a peptide commonly associated with food intake, and several stimuli for drinking behavior. These studies provide additional evidence that ghrelin can affect food and water intake in opposite directions: stimulating food intake while attenuating water intake under some dipsogenic conditions.

Experiments 1 and 2 examined the effect of ghrelin on water intake following exposure to chronic dipsogens. Both of these experiments demonstrated a predictable increase in food intake by ghrelin-treated animals, but unlike our observations in Experiments 3 and 4, ghrelin had no effect on water intake. Although these data show consistent differences in the drinking and feeding responses to ghrelin, the data generated in Experiment 1 are clearly inconsistent with the anti-dipsogenic effect of ghrelin in water-deprived rats reported by Hashimoto *et al.* [21]. It is difficult to reconcile this inconsistency, but differences between Wistar and Sprague Dawley rats might account for the apparent discrepancy. Although a direct comparison of the earlier findings with our experiments is difficult because the previous report only shows data from single times using a similar dose of ghrelin as used here, it is unlikely that additional times would change the overall finding of the previous analysis. It should be noted, however, that other researchers have had difficulty demonstrating an anti-dipsogenic effect of ghrelin in water-deprived animals (Willis K. Samson, *personal communication*). Thus, it appears that the effect of ghrelin on water intake stimulated by deprivation is, at least, less reliable than expected.

Although ghrelin did not affect water intake in the relatively chronic conditions explored by Experiments 1 and 2 (24-hr water deprivation or replacing drinking water with 2.5% NaCl), it had clear anti-dipsogenic effects in Experiments 3 and 4. In these experiments, ghrelin attenuated water intake after administration of peripheral hypertonic saline or central AngII, highlighting the interaction between systems regulating food and water intake. The observed hyperphagia stimulated by ghrelin demonstrates the efficacy of the exogenous peptide and strongly suggests that the decreases in water intake were not due to general malaise or inactivity.

Although the present data indicate that ghrelin inhibits water intake under certain conditions, it is possible that the effect is secondary to the ghrelin-induced hyperphagia. This possibility is unlikely, however, because food intake was greater in ghrelin-treated animals than their controls in all dipsogenic conditions whereas differences in water intake were observed only in some. Nevertheless, we tested this possibility by examining the time course of food and water intake by animals treated with ghrelin alone or ghrelin and one of three dipsogenic conditions: central AngII and peripheral hypertonic saline (both of which were susceptible to ghrelin-induced attenuation of water intake) and 24-hr water deprivation (where we failed to observe an effect of ghrelin on water intake). Our analysis revealed several key points. First, consistent with the findings from our other experiments, water intake was greater in animals deprived of water for 24 hr (a condition in which ghrelin failed to decrease water intake in

Experiment 1) than animals given peripheral injection of hypertonic saline or AngII (conditions in which ghrelin decreased water intake in Experiments 3 and 4, respectively). Although these findings are consistent with our other experiments, we recognize that the direct comparison between ghrelin and vehicle is not present here, thus limiting the comparative value. Second, the majority of water intake occurred during the first 30 min, a period within which relatively little food was consumed. The lack of a systematic difference in the patterns of intake that relates to the differences in the anti-dipsogenic potency of ghrelin observed in earlier experiments strongly suggests that differences in food intake do not account for ghrelin-induced differences in drinking. It remains possible, however, that drinking is more susceptible to attenuation by increased food intake under some conditions than others, but given the timing of the food and water intakes observed here, we believe this is unlikely.

Given the lack of evidence that the differences in water intake were secondary to ghrelin-induced food intake, we must consider other explanations for the observed differences in the anti-dipsogenic potency of ghrelin under the conditions tested. Possible explanations include 1) a fundamental difference in the mechanisms that cause drinking after relatively acute or chronic stimuli, 2) the ability of ghrelin to inhibit drinking stimulated by signals related to osmotic or hypovolemic thirst, but not both, or 3) differences in motivation to drink. The results of Experiment 5 would argue against the first and second of these possibilities because the lack of an effect of ghrelin on drinking after combining acute stimuli resembled our findings from experiments using a single, more chronic stimulus. Additional arguments against the second possibility are found in earlier studies of the physiology of dehydration. Specifically, the stimulus to drink after relatively short-term water deprivation or replacement of drinking water with 2.5% NaCl appears to be mostly osmotic, rather than volemic [24–26]. Thus, it is not likely that differences in the duration of the stimuli or the affected fluid compartment account for the differences in the anti-dipsogenic potency of ghrelin.

It is tempting to speculate that the observed differences in the anti-dipsogenic effects of ghrelin somehow relate to the degree of motivation to drink under the different conditions. Although the available data are limited, there is some support for this idea. For example, Rolls *et al.* [27] showed that rats deprived of water for 24 hr were more tolerant to quinine in their drinking water than rats injected with doses of AngII similar to those used in the present experiments. Thus, there is evidence that the motivation to drink is, in fact, stronger after 24-hr water deprivation than after acute injection of 10 ng AngII. This difference could certainly account for the discrepant anti-dipsogenic potency of ghrelin, at least as it relates to the groups of animals subjected to 24-hr water deprivation or injection of AngII. Nevertheless, further and more specific measures of motivation to drink under each of the dipsetic stimuli used here would be an important step toward clarifying the role of motivation in the observed differences.

More generally, the present data are useful in beginning to understand the interaction between the circuits that mediate feeding and drinking behaviors. In recent years there has been evidence of interplay between brain sites typically attributed to drinking behavior, such as the SFO, and feeding-related peptides [17], but the specifics of this overlap remain uncertain and there are several inconsistencies that remain to be resolved. It is notable, for example, that Pulman *et al.* found SFO neurons that responded to ghrelin *in vitro* [17], but Hashimoto *et al.* failed to detect ghrelin-induced Fos expression in this structure [21]. Although a role for the SFO in the effects observed here cannot be ruled out, it is equally possible that the anti-dipsogenic effects of ghrelin occur through structures already recognized as feeding-related targets of ghrelin, such as the arcuate and paraventricular hypothalamic nuclei.

The present experiments demonstrate that ghrelin reduced water intake under certain circumstances, especially when drinking was induced by a single acute stimulus; however, ghrelin appears to have no effect on water intake generated by chronic dipsetic stimuli or

combination of acute dipsogens that may produce a stronger motivation to drink. Moreover, no patterns in food or water intake were observed that could explain the differential anti-dipsogenic effects of ghrelin. The data presented here provide a behavioral perspective that complements previous anatomical and neurophysiological findings demonstrating overlap between circuits controlling feeding and drinking.

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Figure 1.

Effect of ghrelin on 4 hr food (A) and cumulative water intake (B) following 24-hr water deprivation. ICV injection of ghrelin stimulated food intake but had no effect on drinking. Data are shown as mean $\pm$ SEM ($n=8$ per treatment group); *, $p<0.05$.

Figure 2.

Effect of ICV ghrelin on 4 hr food (A) and cumulative water intake (B) after drinking water was replaced with 2.5% NaCl for 24 hr. Although ghrelin reliably increased food intake, water intake was similar between treatment groups. Data are shown as mean $\pm$ SEM ($n=6-7$ per treatment group); *, $p<0.001$.

Figure 3.

Four-hr food (A) and cumulative water intake (B) after SC injection of hypertonic saline and ICV administration of ghrelin. Ghrelin reliably increased food intake and attenuated water intake. Data are shown as mean $\pm$ SEM ($n=5$ per treatment group); *, $p<0.01$.

Figure 4.

Effect of ICV ghrelin on 4 hr food (A) and cumulative water intake (B) after central administration of AngII. Ghrelin increased food intake in animals, regardless of AngII condition. Animals treated with AngII showed increased water intake that was attenuated by concomitant administration of ghrelin. Data are shown as mean $\pm$ SEM ($n=7-8$ per treatment group); asterisks are used to indicate differences from the veh/veh group in panel A and differences from all other treatment groups in panel B ($p<0.001$).

Figure 5.

ICV ghrelin administration following SC injection of hypertonic saline and central injection of AngII increased 4 hr food intake (A) but did not have an effect on cumulative water intake (B). Data are shown as mean $\pm$ SEM ($n=7$ per treatment group); *, $p<0.05$.

Figure 6.

Non-cumulative food (A) and water (B) intake by animals receiving ghrelin alone or ghrelin and one of three dipsogenic stimuli ($n=4-5$ per treatment group). The legend shown in panel B applies to both panels, but was omitted from panel A for clarity. A main effect of treatment was found for food intake ($p<0.01$). Analysis of water intake revealed main effects of time ($p<0.001$) and treatment ($p<0.001$), as well as a significant interaction ($p<0.001$). Details of the results of statistical analysis and post-hoc comparisons are provided in the results section. The data are presented as mean $\pm$ SEM.

Figure 7.

The non-cumulative intake data from experiment 6 are presented here as percent of total intake at each time measured (mean; error bars have been omitted for clarity). The data from animals receiving ICV ghrelin only (A), peripheral hypertonic saline and ICV ghrelin (B), 24-hr water deprivation followed by ICV ghrelin (C), and ICV AngII and ICV ghrelin (D) reveal no systematic differences in the patterns of intake that account for the different effects of ghrelin in different dipsogenic conditions.