Ghrelin Signaling in the Ventral Hippocampus Stimulates Learned and Motivational Aspects of Feeding via PI3K-Akt Signaling

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Background: The stomach-derived hormone ghrelin drives higher-order feeding processes related to food reward and food seeking via central nervous system signaling at its receptor (GHSR1A). The specific nuclei mediating these effects are only partially understood. Here, we use a rat model to examine whether ghrelin signaling in the ventral subregion of the hippocampus (VHPC), a brain substrate of recent interest in energy balance control, affects learned and motivational aspects of feeding behavior.

Methods: The effects of VHPC ghrelin administration were examined on feeding-relevant behavioral paradigms, including meal pattern analysis, operant lever pressing for sucrose, and conditioned stimulus-induced feeding. The intracellular signaling and downstream neuronal pathways stimulated by VHPC GHSR1A activation were assessed with immunoblot analysis and behavioral pharmacology.

Results: Ghrelin delivery to the VHPC but not the dorsal hippocampus increased food intake primarily by increasing meal frequency. Intra-VHPC ghrelin delivery also increased willingness to work for sucrose and increased spontaneous meal initiation in nondeprived rats after the presentation of a conditioned stimulus that previously signaled meal access when the rats were food-restricted. The food intake enhancing effects of VHPC ghrelin were blocked by co-administration of a phosphoinositide 3-kinase (PI3K) inhibitor (LY294002). Immunoblot analyses provided complementary support for ghrelin activated PI3K-Akt signaling in the VHPC and revealed that this activation is blunted with high-fat diet consumption. Other immunoblot results show that VHPC GHSR1A signaling activates downstream dopaminergic activity in the nucleus accumbens.

Conclusions: These findings illuminate novel neuronal and behavioral mechanisms mediating ghrelinergic control of cognitive aspects of feeding control.

Key Words: Dopamine, GHSR, learning, memory, nucleus accumbens, obesity, reward

Ghrelin is synthesized by gastric endocrine cells and is the only known circulating hormone that increases feeding (1). Central nervous system (CNS) ghrelin signaling stimulates food intake by augmenting appetitive (e.g., food-seeking) (2) and rewarding aspects of feeding (3), yet the neurons and the neural pathways mediating these effects are not completely understood. Investigation of the specific nuclei mediating the food intake regulatory effects of ghrelin has largely focused on hypothalamic (arcuate nucleus, paraventricular nucleus) (4–7), caudal brainstem (nucleus tractus solitarius) (8,9), and midbrain (ventral tegmental area [VTA]) (10–13) nuclei. The ghrelin receptor (GHSR1A) is also expressed in other brain regions, including the hippocampal formation (dentate gyrus and CA1/CA3 regions of the hippocampus) (14,15). Circulating ghrelin reaches the hippocampus where it binds to neurons and promotes dendritic spine synapse formation and long-term potentiation (16). Signaling by GHSR1A in the hippocampus is functionally relevant to learning and memory function, as genetic deletion of ghrelin (16) or its receptor (2) impairs hippocampal-dependent spatial memory paradigms, whereas direct administration of ghrelin to the hippocampus improves memory consolidation for the location of aversive reinforcement (17).

It is unknown whether ghrelin signaling in the hippocampus contributes to food intake and learned appetitive behaviors. The hippocampus is traditionally associated with visuospatial and declarative memory processes (18); however, several recent findings from human and animal models also highlight this brain region in the control of food intake regulation (see [19–22] for reviews). Anorectic control of feeding by the ventral subregion of the hippocampus (VHPC) (anterior hippocampus in primates), which monosynaptically projects to hypothalamic “feeding centers” (23), is directly supported by two recent reports: 1) neurotoxic VHPC lesions increase food intake and body weight in rats (24), and 2) VHPC delivery of the adipose tissue-derived hormone leptin suppresses food intake and learned behaviors related to food procurement (25). Here, we examine the hypothesis that the VHPC also contributes to the mediation of orexigenic (food intake stimulatory) aspects of feeding via ghrelin signaling. Results showed that VHPC GHSR1A stimulation potently increases feeding. The “higher-order” mechanisms (e.g., learned and motivational aspects of feeding) mediating these effects were assessed with various behavioral paradigms, including meal pattern analysis, willingness to work for palatable food (progressive ratio [PR] operant responding), and the initiation of meals induced by conditioned cues previously associated with food reward.

We also examine the downstream neuronal pathways and intracellular signaling mechanisms mediating VHPC ghrelin effects on food intake. The VHPC neurons project directly to the nucleus accumbens (NAc) of the mesolimbic reward system (MRS) (26,27). Central (intracerebroventricular [ICV]) administration of ghrelin elevates dopaminergic activity in the NAc (28). Present experiments employ protein immunoblot analyses to examine the hypothesis that VHPC GHSR1A stimulation influences downstream catecholamine signaling in the NAc. We also examine the intracellular signaling pathways mediating food intake elevations by VHPC ghrelin signaling. Recent findings show that ghrelin activates the phosphoinositide 3-kinase (PI3K)-Akt intracellular signaling in neurons

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(29,30). Unknown is whether feeding effects driven by VHPC GHSR1A stimulation require PI3K-Akt signaling.

Methods and Materials

Animals and Drugs

Adult male Sprague-Dawley rats (Charles River, Wilmington, Massachusetts) (300–500 g during experimental procedures) housed individually under a 12-hour light/dark cycle had ad libitum access to chow (LabDiet 5001, LabDiet, St. Louis, Missouri) and water except where noted. All procedures conformed to and received approval from The University of Pennsylvania Animal Care and Use Committee.

Ghrelin (Bachem, King of Prussia, Pennsylvania) was dissolved in artificial cerebrospinal fluid; the PI3K inhibitor LY294002 (EMD Millipore; Merck KGaA, Darmstadt, Germany) was dissolved in dimethyl sulfoxide (DMSO). Volumes for injections were 100 nL/hemisphere for parenchymal (via Harvard Apparatus infusion pump; Harvard Apparatus, Holliston, Massachusetts) and 1 μL for ICV.

Cannula Implantation

Under ketamine (90 mg/kg), xylazine (2.7 mg/kg), and acepromazine (.64 mg/kg) anesthesia and analgesia (Metacam; Boehringer Ingelheim Vetmedica, St. Joseph, Missouri) (2 mg/kg), guide cannulae (Plastics One, Roanoke, Virginia) (26-guage) cemented to the skull with jewelers screws were implanted at the following coordinates for VHPC placement: 4.9 mm anterior/posterior (A/P), 4.8 mm medial/lateral (M/L), 6.1 mm dorsal/ventral (D/V); for dorsal hippocampus (DHPC) placement: 3.5 mm A/P, 2.5 mm M/L, 2.0 mm D/V; for lateral ICV placement: .9 mm A/P, + 1.6 mm, M/L, 2.8 mm, D/V. Injectors for drug administration projected 2 mm beyond guide cannula for VHPC and ICV injections and 1 mm for DHPC. Cannula placements for VHCP and DHPC were assessed postmortem through anatomical verification of the position of 100-nL pontamine sky blue injections in coronal sections. Only animals with ink observed within the targeted region (VHPC CA regions) were included in data analyses. A representative VHPC injection site is shown in Figure S1 in Supplement 1. The number of animals excluded on the basis of incorrectly targeted cannula ranged between 0 and 2 for each experiment. Anatomical positions of lateral ICV injection sites were evaluated 1 week post-surgery by measurement of the cytoglucopenia-induced sympatho-adrenal mediated glycemic effect resulting from 210 μg (2 μL) of 5-thio-D-glucose (31,32).

Signaling Analysis

Tissue Collection. The VHPC (CA regions) and NAc tissue from ad libitum chow-fed rats was prepared as described (31,33). Briefly, after pharmacological treatments rats were sacrificed by decapitation. As previously described (34), brains were rapidly removed, and bilateral tissue punches were taken from the VHPC and NAc with stainless steel tubing (inside diameter 2.3 mm) from 2-mm coronal brain block sections. Tissue was flash frozen in isopentane and stored at −80°C.

Immunoblotting. Lysates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluouride membranes for immunoblot analysis as previously described (31,35). Immunoreactivity was visualized with enhanced chemiluminescence (BioRad, Hercules, California; Chemidoc XRS). Phosphorylated PI3K p85 (Tyr458) and PI3K p85 antibodies (Cell Signaling, Danvers, Massachusetts) were used to evaluate PI3K activity normalized to total PI3K. Phosphorylated AKT (Ser471) (Cell Signaling) and Anti-Akt (Pierce Antibodies; Thermo Scientific, Rockford, Illinois) antibodies were used to evaluate Akt activity normalized to total Akt. Phosphorylated p44/42 mitogen-activated protein kinase (MAPK) antibody (Thr202/Tyr204) was used to assess MAPK signaling normalized to total p44/42 MAPK (Cell Signaling). Phosphorylated tyrosine hydroxylase (pTH) antibodies (Cell Signaling) were used to evaluate tyrosine hydroxylase (TH) activity normalized to total TH. Blots were quantified with densitometry analysis using National Institutes of Health software (Image J).

Procedures

Experiment 1: Food Intake After VHPC and DHPC Ghrelin. Rats with either VHPC ($n = 12$) or DHPC ($n = 12$) cannulae were given bilateral injections of 0, 7.5, 75, or 750 pmol ghrelin (total doses: 15, 150, 1500 pmol) immediately before light onset. Treatments were separated by 2–3 days using a counterbalanced within-subjects design. Chow intake was recorded at 1 hour, 3 hours, and 5 hours (spillage accounted for).

Ghrelin dose selection for Experiments 1 and 2 was based on the literature. Previous studies show that parenchymal ghrelin doses of approximately 300 pmol seem to be required for intake effects when delivered to various hypothalamic nuclei (lateral hypothalamus, anterior hypothalamus) (4). After administration of ghrelin to the NAc and VTA, 100 pmol (36) and 150 pmol (12) seem to be required for increasing intake, respectively. Lower doses of ghrelin are effective for increasing feeding when delivered to the nucleus tractus solitarius (8) or the arcuate nucleus (4) (10 or 30 pmol, respectively).

Experiment 2: VHPC Ghrelin Effects on Meal Pattern Parameters. The VHPC injections (0, 75, or 150 pmol ghrelin) were given to rats ($n = 13$) immediately before light onset with a within-subjects design. Cumulative intake was measured with a custom-built automated feeding system. Individually housed rats had access to a food cup on a load cell circuit that communicated with an interface and computer with customized software (LabVIEW, National Instruments, Austin, Texas). The weight of the food cup was measured every 10 sec, enabling assessment of meal parameters. Meals were defined as an episode of feeding in which at least .25 g was ingested, with meal termination criterion as the beginning of a pause in ingestion of at least 10 min (37). Data were objectively calculated with a custom Microsoft Excel macro.

Experiment 3: Operant Responding (PR Schedule) for Sucrose After VHPC Ghrelin. Rats ($n = 6$) were given operant lever press training for sucrose reinforcement as previously described (38). Rats were given daily chow rations to maintain approximately 85% of an ad libitum body weight established before training. Training was carried out over 6 days with a 1-hour session each day in conditioning boxes (Med Associates; MedPC IV Software, St. Albans, Vermont). During the first 2 days a fixed ratio (FR1) autoshaping procedure was employed (each lever press earned a 45-mg sucrose pellet; a free sucrose pellet dispensed every 600 sec that elapsed without reinforcement). The animals then received 2 days of FR1 schedule with no autoshaping component and then 2 days of FR3 training. For all procedures the right lever was the “active” lever; a left “inactive” lever served as a control for nonconditioned elevations in responding.

The rats were given two tests (within-subjects design, separated by 2 days) with a PR reinforcement schedule. The VHPC injections (vehicle or 150 pmol ghrelin) were given 1 hour before each test session. The response requirement of the PR schedule increased progressively as previously described (38). The breakpoint for each animal was defined as the final completed requirement that preceded a 20 min period without earning a reinforcer.

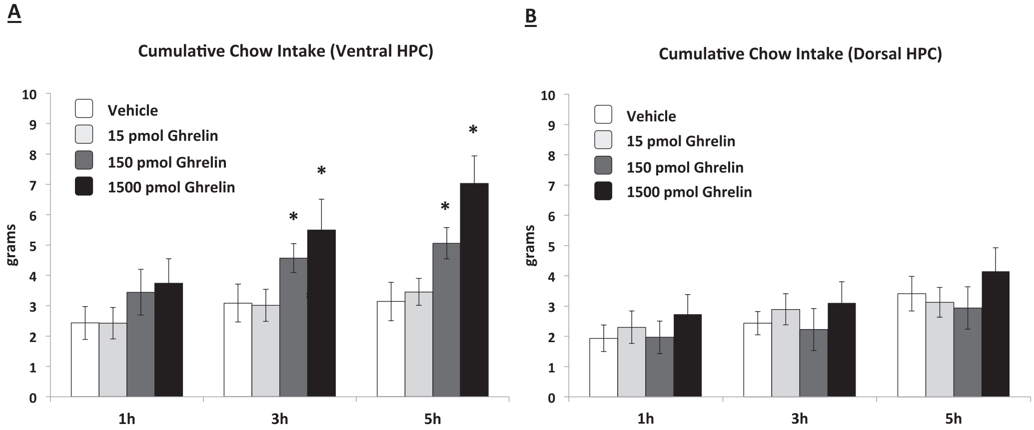

Figure 1. Cumulative chow intake after ventral hippocampus (HPC) **(A)** or dorsal HPC **(B)** administration of ghrelin. Ventral but not dorsal HPC ghrelin delivery stimulated food intake relative to vehicle treatment. Data are mean ± SEM; *$p < .05$ versus vehicle. h, hour.

Experiment 4: Stimulus-Induced Feeding by VHPC Ghrelin. Previous studies show that discrete stimuli (lights, tones) previously paired with meal access when rats were food restricted would later stimulate increased eating when the rats were food-sated (39–43). We hypothesized that VHPC ghrelin signaling would increase this type of "cue-potentiated feeding." We developed a paradigm (modified from [44]) in which discrete cues were paired with meal access in food-deprived rats (Stimulus+); the presentation of another discrete cue had no consequence (Stimulus−). The paradigm was designed to be below threshold for cue-potentiated feeding at baseline (i.e., weak effect of cues on feeding in the absence of pharmacological stimulation).

Rats (*n* = 13) were maintained on a high-fat diet (HF) (60% kcal fat; Research Diets D12492, New Brunswick, New Jersey) for 5 days before training. All training and testing procedures took place during the dark cycle. Ten training days were given where they received five meals (HF diet) distributed across the first 8 hours of the dark cycle. The total kcal of the five meals was equal to 70% of an ad libitum 24-hour intake established before training for each rat. On half of the training days the rats received five presentations of a 2.5-min auditory/visual stimulus compound (Stimulus+) followed immediately by meal access. For the other half, a different auditory/visual stimulus compound (Stimulus−) was presented five times, and the five meals were delivered at random times. The two stimuli were: 1) a 2.5-min 1500-hz tone combined with a dim light coming from one side of the room, and 2) a 2.5-min white noise combined with a dim light coming from the other side of the room. The order of training days and stimulus assignments were counterbalanced.

After training the rats were returned to ad libitum HF diet feeding. Cue-potentiated feeding was determined as a meal initiated within 3 min of stimulus onset (within 30 sec of stimulus offset). The rats were housed in the automated feeding apparatuses (described in the preceding text), so that meal initiation could be determined with temporal specificity in relation to stimuli presentation. To confirm that this paradigm was subthreshold for cue-potentiated feeding at baseline, stimulus tests with five presentations of each stimulus were conducted on days 5, 6, and 7 of ad libitum feeding. These tests revealed no difference between the number of meals that followed Stimulus+ versus Stimulus– (data not shown). A pharmacological test was then given on days 9 and 15 of ad libitum feeding where the rats were given VHPC ghrelin (150 pmol) or vehicle injections (order counterbalanced) immediately before dark onset. The rats were then given five presentations of each stimulus across the subsequent 6 hours.

Experiment 5a: Ghrelin-Induced VHPC PI3K-Akt Signaling. Rats (*n* = 26) were maintained on chow or a "Western diet" (41% kcal from fat; Research Diets D12079B) for 4 weeks. The rats from each diet group were subdivided (matched for body weight within each diet group) to receive lateral ICV ghrelin (3 nmol; dose selected to be effective for robustly increasing intake after ICV delivery [45]) or vehicle injections 60 min before VHPC tissue harvest. Immunoblot analysis (PI3K, Akt, and p44/42 MAPK) was carried out as described in the preceding text.

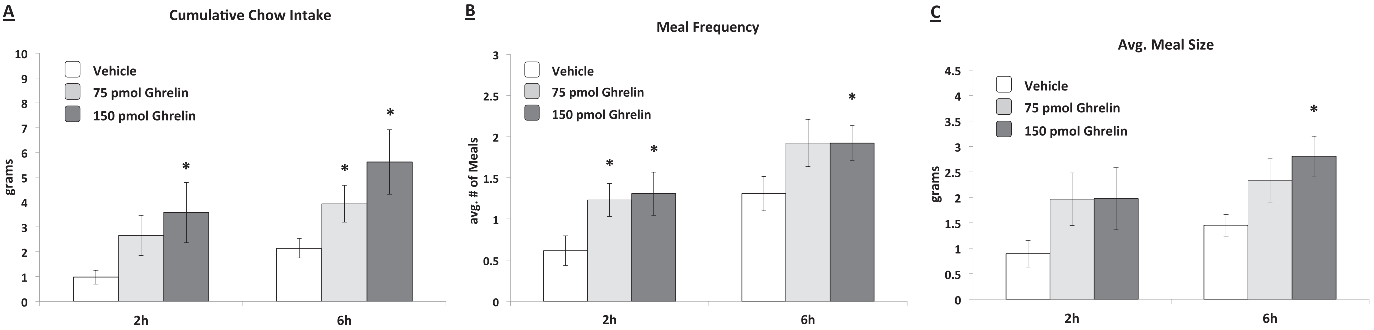

Figure 2. Cumulative chow intake **(A)**, average meal frequency **(B)**, and average meal size **(C)** after ventral hippocampus ghrelin delivery. Both 75- and 150-pmol ghrelin increased cumulative food intake and meal frequency; 150-pmol ghrelin also increased average 6-hour (h) meal size relative to vehicle treatment. Data are mean ± SEM; *$p < .05$ versus vehicle.

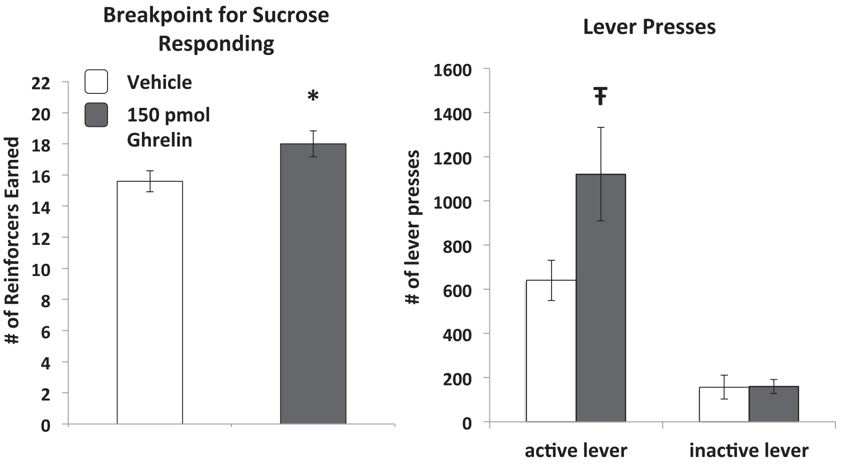

Figure 3. Ventral hippocampus ghrelin increased breakpoint operant responding for sucrose in a progressive ratio reinforcement test. No ghrelin treatment-based differences in lever pressing were observed for the inactive lever. Data are mean ± SEM; *$p < .05$ versus vehicle; Ŧ$p < .07$ versus vehicle.

Experiment 5b: Requirement of PI3K-Akt Signaling for VHPC Ghrelin-Stimulated Feeding. With a four-treatment within-subjects design, rats ($n = 13$) received two sets of bilateral VHPC injections on each treatment day (injections approximately 30 min apart; treatments separated by 2–3 days). The first injection was the PI3K inhibitor LY294002 (.2 nmol) or its vehicle, whereas the second injection (immediately before light onset) was ghrelin (150 pmol) or its vehicle.

Experiment 6: VHPC Ghrelin Effects on NAc Catecholamine Signaling. Rats ($n = 18$) were divided into four groups (4–6/group) to receive VHPC vehicle or ghrelin (150 pmol) injections either 120 min or 60 min before tissue harvest. These time points were chosen on the basis of previous work demonstrating increased NAc dopamine (DA) signaling after ghrelin administered to the VTA (46). The NAc tissue harvest and immunoblot analysis (pTH/TH) were carried out as described in the preceding text. Previous research has used immunoblot pTH analysis to assess dopaminergic NAc signaling (47,48).

Statistical Analysis

All statistical analyses employed repeated measures analysis of variance, except for Experiments 5a and 6 (one-way analysis of variance). Newman-Keuls post hoc tests were used to compare individual treatments for all experiments that involved more than two treatments. The α level for significance was .05. Statistical analyses were conducted with Statsoft software (Statistica V10; Statsoft, Tulsa, Oklahoma).

Results

Experiment 1

Ghrelin delivered to the VHPC significantly increased food intake at 3 hours and 5 hours compared with vehicle injection for the two higher doses (Figure 1A) (*p* values vs. vehicle < .05). The DHPC ghrelin injections had no effect on food intake for all doses examined (Figure 1B).

Experiment 2

Ghrelin injections in the VHPC increased cumulative food intake for both the 75-pmol and the 150-pmol doses (Figure 2A). This increased feeding seemed to be based on increased meal frequency for both doses (Figure 2B) (*p* values < .05 vs. vehicle), whereas only the 150-pmol dose significantly increased meal size relative to vehicle treatment (Figure 2C).

Experiment 3

As shown in Figure 3, VHPC ghrelin increased breakpoint responding for sucrose during the PR test relative to vehicle treatment ($p < .05$). This effect was based on elevated active lever pressing, whereas pressing of the inactive lever was not influenced by VHPC ghrelin.

Experiment 4

Consistent with the results of the cue-potentiated feeding tests that were conducted before the VHPC ghrelin test, there was no baseline (after vehicle administration) cue-potentiated feeding effect. However, relative to vehicle treatment, VHPC ghrelin (150 pmol) significantly elevated the number of meals that followed presentation of the Stimulus+ but not after the Stimulus– ($p < .05$ for ghrelin Stimulus+ vs. all 3 other treatments) (Figure 4). Analysis of the average size of each stimulus-induced meal revealed no significant differences with regard to stimulus or drug (data not shown).

Experiment 5

Experiment 5a. Comparison of the chow vehicle-treated group with the Western diet vehicle-treated group revealed no

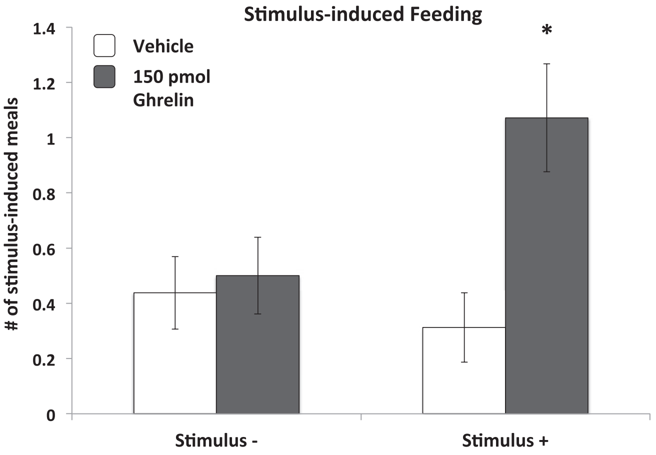

Figure 4. Ghrelin delivered to the ventral hippocampus in ad libitum rats increased spontaneous meals initiated by a discrete cue that was previously associated with meal access when the rats were food deprived (Stimulus+), whereas this effect was not observed for a cue that was never paired with meal access (Stimulus−). Data are mean ± SEM; *$p < .05$ versus all other treatments.

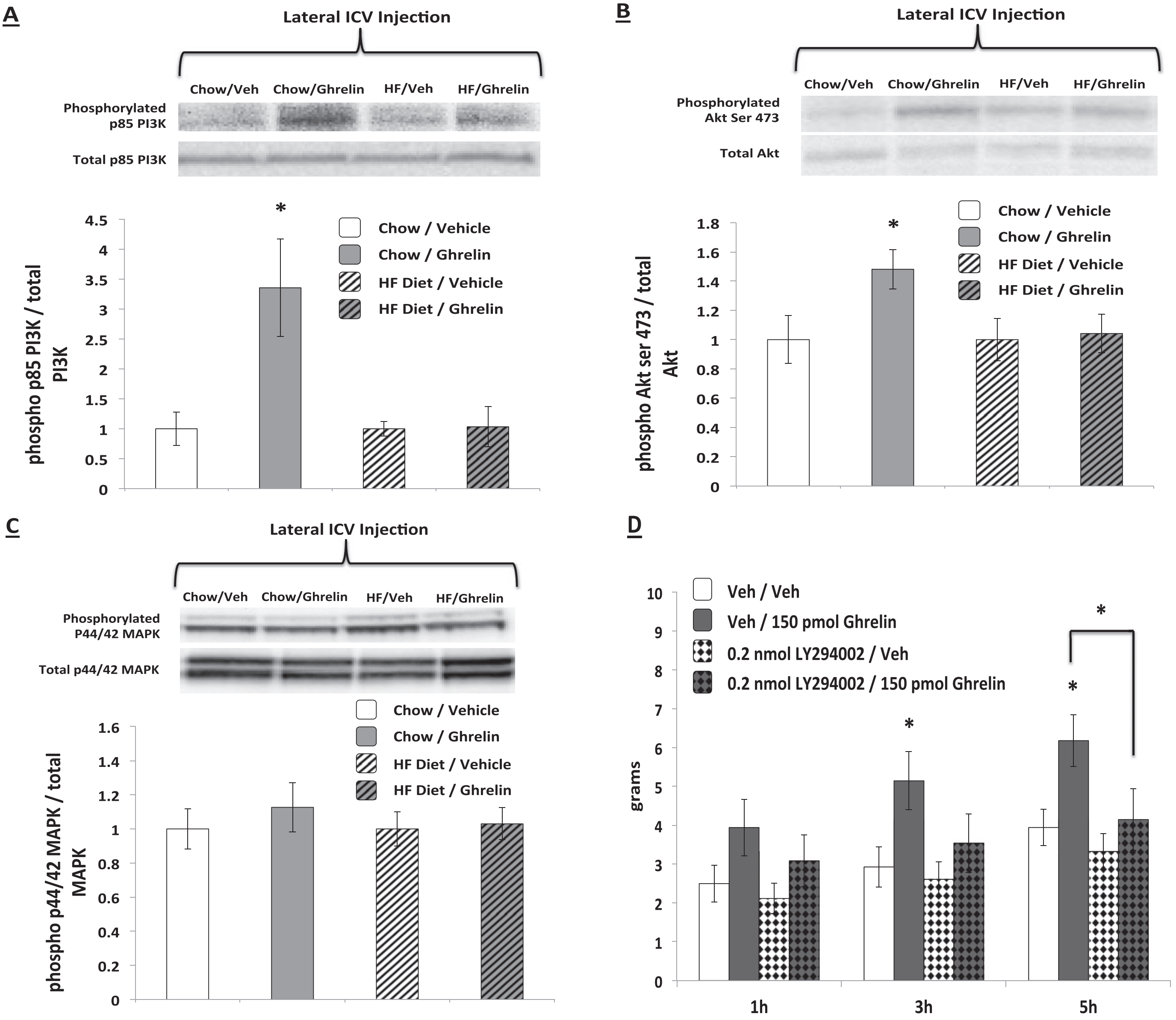

Figure 5. Ghrelin (delivered lateral intracerebroventricular [ICV]) activated phosphoinositide 3-kinase (PI3K) **(A)** and Akt **(B)** signaling in the ventral hippocampus in chow-fed but not Western diet-fed rats, whereas p44/42 mitogen-activated protein kinase (MAPK) signaling was not elevated by ghrelin **(C)**. The food intake stimulatory effects of ventral hippocampus ghrelin were blunted with co-administration of the PI3K inhibitor LY294002 at 3 hours and 6 hours after injections **(D)**. Data are mean ± SEM; $^*p < .05$ vs. vehicle (Veh). HF, high-fat.

significant differences for PI3K, Akt, or p44/42 MAPK activation. Thus, data are expressed as percentage of the vehicle-treated groups separately for each diet group to better illustrate ghrelin-induced increased signaling within each diet group. Ghrelin injected ICV (3 nmol) significantly increased VHPC PI3K (Figure 5A) and Akt activation (Figure 5B) in chow-fed but not Western diet-fed rats. Activation of p44/42 MAPK signaling in the VHPC was not augmented by ghrelin (Figure 5C). Chow-fed vehicle- and ghrelin-treated rats used for immunoblot analysis weighed 498.4 (± 30.6) and 490.4 (± 30.0) g, respectively. Western diet-fed vehicle-treated and ghrelin-treated rats weighed 592.9 (± 27.1) and 585.9 (± 8.6) g, respectively.

Experiment 5b. Ghrelin-stimulated food intake at 3 hours and 5 hours after injections was blocked with pretreatment of the PI3K inhibitor LY294002 (Figure 5D). At 3 hours, DMSO/ghrelin treatment increased food intake relative to DMSO/artificial cerebrospinal fluid treatment ($p < .05$), whereas LY294002/ghrelin treatment was not significantly different from any other treatment. At 5 hours, DMSO/ghrelin treatment produced significantly greater food intake compared with all other treatments ($p < .05$); a significant drug interaction was also obtained at 5 hours ($p < .05$).

Experiment 6

Relative to total TH, pTH levels were not significantly different between the two vehicle groups (60 and 120 min); thus, the vehicle groups were combined for subsequent analyses and for Figure 6. Ghrelin (150 pmol) injected in the VHPC significantly increased pTH in the NAc at 60 min after injections ($p < .05$) (Figure 6).

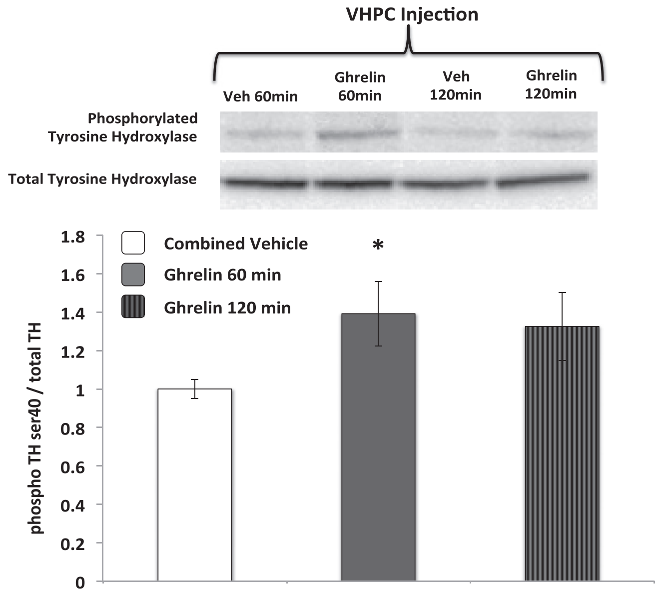

Figure 6. Ventral hippocampus (VHPC) ghrelin administration increased phosphorylated tyrosine hydroxylase (TH) in the nucleus accumbens at 60 min after injections. Data are mean ± SEM; $^{*}p < .05$ vs. vehicle (Veh).

Discussion

Research examining energy balance control by central nervous system ghrelin signaling has focused primarily on brain regions traditionally associated with homeostatic aspects of food intake (e.g., hypothalamus, caudal brainstem) and more recently on MRS nuclei such as the VTA (12,13,36) and the NAc (13,36). Here, we establish the VHPC, a brain region linked with emotional and motivational memory processes, as a novel site regulating learned and rewarding orexigenic aspects of feeding by ghrelin signaling. Ghrelin administration to the VHPC potently stimulated food intake in rats, the latency of which was similar to previous studies administering ghrelin ICV (49,50). By contrast, ghrelin administration to the DHPC, an area associated with the control of learning/memory function related to visuospatial processing (51,52), was without effect on feeding. These findings complement our previous work showing that food intake and learned aspects of feeding are suppressed by VHPC leptin signaling (25).

The increased feeding response by VHPC GHSR1A stimulation was largely mediated by increased meal frequency, whereas higher doses increased both meal frequency and size. The meal size effect suggests that ghrelin signaling in the VHPC functions, in part, to reduce the effectiveness of satiation signals that arise during a meal, a notion consistent with recent data showing that the hippocampus is activated by gastric distention (53) and intragastric nutrients (54). We hypothesized that the increased meal frequency effect was based on VHPC ghrelin signaling augmenting spontaneous feeding episodes that arise in response to the presence of conditioned food-related environmental cues. To examine this possibility, we developed a "cue potentiated feeding" paradigm in which food-restricted rats were trained such that a discrete stimulus signaled meal access (Stimulus+) whereas another stimulus did not (Stimulus−). Other researchers have developed similar paradigms in which food-related cues stimulate excessive food intake in food-sated rats that would not otherwise eat (39,41,43,44). Results showed that VHPC ghrelin increased meal initiation that followed the presentation of the Stimulus+ but not the Stimulus−. These findings provide novel information about the neuroendocrine systems mediating environmental cue-driven feeding. The relevance of these findings to human obesity is underscored by a recent report estimating that a substantial portion of increased per capita caloric intake since the 1970s is based on increased number of eating occasions (meals, snacks) (55), an effect that is potentially influenced by the increased pervasiveness of environmental cues associated with energy-dense, rewarding food (56). That GHSR1A activation in the VHPC increased stimulus-induced meal initiation in ad libitum-fed animals suggests a nonhomeostatic function (food intake driven by factors other than metabolic need) for this system. Future work could examine whether this type of cue-driven feeding effect is unique to the VHPC or also involves GHSR signaling in other brain regions thought to control homeostatic (e.g., hypothalamus, brainstem) and nonhomeostatic (VTA) aspects of feeding.

Signaling by GHSR1A modulates rewarding aspects of feeding in paradigms that assess motivation to obtain palatable food, such as conditioned place preference (3) and PR lever pressing (36,57). These motivational/reward augmenting effects likely involve altered dopaminergic signaling in the MRS structures, as previous findings show that intra-VTA ghrelin increases operant responding for sucrose (36) and central or peripheral ghrelin stimulates VTA/NAc DA signaling (assessed from electrophysiology [12] and microdialysis [12,28]). Present results expand knowledge of the reward-associated neural circuitry mediating the effects of ghrelin on feeding by showing that VHPC GHSR1A signaling elevates willingness to work for sucrose in a PR operant lever pressing paradigm and VHPC ghrelin delivery elevates pTH expression in the NAc 60 min after administration, likely indicating enhanced DA release from local terminals arising from the VTA. These findings are consistent with previous results showing that the VHPC projects directly to the NAc shell (26,27) and glutamatergic signaling in the VHPC has an acute stimulatory effect on NAc DA release via a polysynaptic pathway (58). The specific neuronal pathways mediating VHPC ghrelin-mediated effects on NAc DA signaling remain to be determined. Furthermore, given that CNS ghrelin signaling modulates the reinforcing properties of other primary reinforcers (e.g., alcohol [59], cocaine [60]), further work is needed to assess whether VHPC ghrelin signaling increases motivation for drugs of abuse. Indeed, several findings link VHPC neuronal activity with behavioral paradigms related to cocaine reward (61,62).

Our results show that feeding effects triggered by VHPC GHSR1A signaling involve intracellular PI3K-Akt signaling, a phenomenon demonstrated by others for hypothalamic leptin receptor signaling (63,64). The GHSR1A is a rhodopsin-like G-protein coupled receptor that triggers intracellular second messengers through the activation of G_q proteins (65). Previous studies have shown that adenosine monophosphate-activated protein kinase (AMPK) is activated in the hypothalamus by ghrelin (66,67). Ghrelin also initiates changes in hypothalamic mitochondrial respiration through uncoupling protein 2 and AMPK-dependent mechanisms (68) and elevates cyclic (c)AMP response-element binding protein activity through a protein kinase A-dependent mechanism (69). Our focus in the present report was on PI3K-Akt signaling, largely based on a recent report demonstrated that ghrelin activates Akt in the dorsal dentate gyrus of the hippocampal formation and that enhanced water maze learning by ghrelin was blocked by dentate gyrus PI3K inhibition (29). Here, we extend these findings in several ways. Results show that the PI3K-Akt pathway is activated in the VHPC, this activation is required for the food intake-enhancing effects of VHPC-directed ghrelin, and activation of this pathway is compromised by intake of a "Western" diet. Others have demonstrated a similar type of "CNS ghrelin resistance" at the neuronal level (reduced activation of hypothalamic neuropeptide Y neurons in diet-induced obese [DIO] mice [70]) and at the behavioral level (ghrelin augmented operant PR responding in normal weight but not DIO mice [71]). Our findings show that diet-induced CNS ghrelin resistance can also occur at the intracellular signaling level. Further study is needed to assess whether other intracellular signaling pathways associated with GHSR1A activity are activated by ghrelin in the VHPC (e.g., AMPK, protein kinase A–cAMP response-element binding protein) and whether DIO blunts the feeding effects of VHPC GHSR1A signaling, a phenomenon demonstrated for leptin signaling in the hypothalamus (72,73). Data from Experiment 4 suggest that the stimulatory effect of ghrelin on cue-potentiated feeding is preserved under certain conditions of HF diet maintenance. However, these rats were maintained on a HF diet for only approximately 3 weeks (vs. 4 weeks for Experiment 5a) and were food restricted for 10 of these days. A more systematic evaluation would be necessary before concluding whether the effects of HF diet intake on GHSR1A intracellular PI3K-Akt signaling are correlated with VHPC GHSR1A "resistance" at the behavioral level.

To our knowledge this is the first report to examine behavioral effects of VHPC GHSR1A activation; however, other researchers have assessed the effects of DHPC ghrelin delivery on various behavioral paradigms. Ghrelin signaling in the DHPC has been shown to improve spatial memory performance in the Morris water maze paradigm (29). Carlini *et al.* (74–76) reported that DHPC-directed ghrelin improves memory consolidation for aversive reinforcement in a step-down inhibitory avoidance paradigm and that this effect is blocked by co-administration of a serotonin reuptake inhibitor. These investigators also demonstrated that 1.5 and 3 nmol ghrelin delivered to the DHPC significantly increased food intake versus vehicle treatment (77). This contrasts with our results, as DHPC ghrelin delivery (at doses up to 1.5 nmol) had no effect on feeding. The reasons for this discrepancy are not clear but could potentially be based on differences in rat strain (Sprague-Dawley vs. Wistar) or injection volume. Our volume/hemisphere (100 nL) was fivefold lower than the volume these investigators employed. Regardless, our results make a strong case that feeding effects by GHSR1A stimulation in the hippocampus are far more potent with VHPC compared with DHPC delivery.

Overall these findings establish the VHPC as a novel site of importance in the stimulation of food intake and other appetitive/rewarding behaviors by CNS ghrelin signaling. Taken together with our previous work (25), present results support the perspective that the VHPC modulates both anorectic and orexigenic processes related to the higher-order control of food intake through detection and processing of circulating energy status-relevant neuroendocrine signals. Results show that VHPC ghrelin signaling stimulates feeding by increasing the ability of environmental food-related cues to stimulate meal initiation and by increasing motivation to work for palatable food. Other results inform about the intracellular signaling and the downstream neuronal pathways mediating these effects. These findings are relevant to human obesity, given the abundance of palatable yet nutritionally deplete foods as well as the abundance of environmental cues that are associated with these foods in modern Western cultures.

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