Ghrelin is an orexigenic and metabolic signaling peptide in the arcuate and paraventricular nuclei

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Submitted 5 November 2004; accepted in final form 5 April 2005

Ghrelin is an orexigenic and metabolic signaling peptide in the arcuate and paraventricular nuclei. Am J Physiol Regul Integr Comp Physiol 289: R353–R358, 2005. First published April 7, 2005; doi:10.1152/ajpregu.00756.2004.—Ghrelin is a 28-amino acid acylated peptide and is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R). The GHS-R is expressed in hypothalamic nuclei, including the arcuate nucleus (Arc) where it is colocalized with neuropeptide Y (NPY) neurons. In the present study, we examined the effects of ghrelin on feeding and energy substrate utilization (respiratory quotient; RQ) following direct injections into either the arcuate or the paraventricular nucleus (PVN) of the hypothalamus. Ghrelin was administered at the beginning of the dark cycle at doses of 15–60 pmol to male and female rats. In feeding studies, food intake was measured 2 and 4 h postinjection. Separate groups of rats were injected with ghrelin, and the RQ (V\(_{\text{CO}_2}/V_{\text{O}_2}\)) was measured using an open circuit calorimeter over a 4-h period. Both Arc and PVN injections of ghrelin increased food intake in male and female rats. Ghrelin also increased RQ, reflecting a shift in energy substrate utilization in favor of carbohydrate oxidation. Because these effects are similar to those observed after PVN injection of NPY, we then assessed the impact of coinciding ghrelin with NPY into the PVN. When rats were pretreated with very low doses of ghrelin (2.5–10 pmol), NPY’s (50 pmol) effects on eating and RQ were potentiated. Overall, these data are in agreement with evidence suggesting that ghrelin functions as a gut-brain endocrine hormone implicated in the regulation of food intake and energy metabolism. Our findings are also consistent with a possible interactive role of hypothalamic ghrelin and NPY systems.

Recent evidence suggests that the gastric peptide plays a particularly important role in the regulation of energy homeostasis and that this role is exerted at the level of the central nervous system (CNS). Central injections of ghrelin elicit food intake (4, 8, 24, 68), and chronic ventricular administration to rodents induces body weight gain and enhanced body fat mass deposition (28, 64). Indeed, ghrelin appears to interact with other peptidergic systems within the hypothalamus, including neuropeptide Y/agouti-related peptide (NPY/AGRP) neurons of the arcuate nucleus (Arc) (25, 61, 67). This latter finding suggests that hypothalamic nuclei may be key sites of action for the peptide. In support of this hypothesis, a limited number of microinjections studies, including our own data, indicate that ghrelin administration into discrete hypothalamic nuclei stimulates appetite and/or energy metabolism (16, 37, 66, 68). Ghrelin’s orexigenic action is comparable to that of NPY in magnitude (62, 68), and similar changes in nutrient partitioning have been reported with neuropeptide Y (NPY) (11–17). Paraventricular nucleus (PVN) injections of NPY evoke a marked increase in respiratory quotient (RQ), independent of its feeding stimulant effects (10–17, 38). Interestingly, compared with peripheral treatment, ghrelin alters food intake and body weight greater than 1,000-fold more potently after central administration (28), consistent with the hypothesis that the peptide influences energy homeostasis predominantly through the stimulation of central mechanisms. Moreover, these effects are observed using physiologically relevant doses (64).

On the basis of such findings, we propose that ghrelin alters appetite and energy metabolism, including the promotion of carbohydrate oxidation and the conservation of fat stores, by an action on hypothalamic circuits mediating positive energy balance. In the present study, we examined the effects of ghrelin administration in two key regions of the hypothalamus, the Arc and PVN, to examine the peptide’s effects on food intake and energy substrate use as measured by indirect calorimetry. We also examined a possible interactive role for ghrelin and NPY on these measures.

MATERIALS AND METHODS

Subjects. Adult male Sprague-Dawley rats weighing 275–325 g at the time of surgery were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed individually in polypropylene cages with free access to standard rodent food (Purina) and water. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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http://www.ajpregu.org

First published April 7, 2005; doi:10.1152/ajpregu.00756.2004.
colony room was maintained at a temperature of 22 ± 2 °C and on a 12:12-h light-dark cycle.

**Apparatus.** Oxygen consumption (O$_2$) and carbon dioxide (CO$_2$) production were measured using an Oxyscan open-circuit indirect calorimeter (AccuScan Instruments, Columbus, OH). Detectors measured O$_2$ and CO$_2$ sequentially across each acryl test chamber. A constant flow rate of 1,500 ml/min was established. Concentrations of the gases were recorded in milliliters per kilogram body weight per minute. RQ was calculated as the volume of CO$_2$ produced (V$_{CO_2}$) divided by the volume of O$_2$ consumed (V$_{O_2}$). Analyzers were calibrated before each use with primary gas standards of high purity (Matheson, New York, NY).

**Surgery.** Chronic indwelling guide cannulas (steel 22-gauge; Plastics One, Roanoke, VA) were implanted in all animals aimed at either the Arc or the PVN. Rats were anesthetized with ketamine (75 mg/kg ip) and xylazine (5 mg/kg ip) and placed in a Kopf stereotaxic apparatus with the incisor bar set at 3.5 mm below the interaural line. Cannulas were implanted 4 mm dorsal to target, as described previously (43) relative to bregma: Arc AP = −2.3 mm, L = −0.3 mm, V = −5.8 mm; PVN AP = −1.8 mm, L = −0.4 mm, V = −4.0 mm. Each implant was secured with acrylic cement, and three stainless screws penetrated the skull. Guide cannulas were fitted with 28-gauge stainless steel stylets to maintain patency. All rats were administered 0.4 mg/kg subcutaneously of the analgesic buprenorphine at the time of surgery and once again 12 h later. Testing began after a postoperative recovery period of 10 days. During this time, food intake and body weights were monitored, and animals were familiarized with the injection and testing procedures.

**Design and procedure.** Male and female rats were injected with ghrelin or vehicle just before the beginning of the nocturnal cycle. Ghrelin was administered at doses of 15, 30, and 60 pmol, in a 0.3-μl volume and dissolved in sterile saline. A repeated-measures design was used with all rats receiving each dose of peptide or vehicle, administered in a randomized order. A minimum of 4 days separated successive testing. In feeding studies (n = 9/group), rats were tested in their home cages and food intake, corrected for spillage, was measured 2 and 4 h postinjection. In metabolic testing (n = 8), identical injection procedures were followed. Immediately after treatment, rats were placed in individual chambers of the metabolic apparatus, where O$_2$ consumption and CO$_2$ production were measured over 4 h. Food and water were not available during this time.

In separate groups of male and female rats with cannulas aimed at the PVN, ghrelin (2.5, 5, or 10 pmol), or vehicle was administered 5 min before injection of NPY (50 pmol) or vehicle. NPY administration coincided with the start of the dark cycle. Feeding (n = 9) and metabolic (n = 8) protocols were identical to the above paradigms, except that food intake and RQ were measured over 2 h postinjection. Again, injections were separated by 4 days with treatments conducted in randomized order. In the control condition, rats received two injections of sterile saline. Ghrelin and NPY coinjections were examined in the PVN alone since only PVN injections of NPY are reported to alter RQ (10–17, 38).

**Statistical and histological analyses.** Data were analyzed using two- and three-way repeated-measures ANOVA followed by post hoc analysis of individual mean comparisons where appropriate. The criterion for statistical significance was P < 0.05. The accuracy of all cannula placements was confirmed via histological assessment as described previously (11, 15). Sections were examined by light microscopy and viewed relative to the stereotaxic atlas of Paxinos and Watson (43). All animals reported here were found to have injector tracts extending into the appropriate target nucleus.

**RESULTS**

Figures 1 and 2 depict the dose-response effects of ghrelin in male and female rats after injection into either the Arc or PVN of the hypothalamus. Ghrelin increased food intake when administered into the Arc of male and female rodents [F(1,14) = 56.6, P < 0.0001]. A similar effect on food intake was observed upon injection into the PVN [F(1,14) = 146.1, P < 0.0001]. Intake was significantly increased at both 2 and 4 h into the dark period. The effect of ghrelin on energy substrate use is illustrated in Fig. 3 and Fig. 4. RQ values are shown in 40 min intervals across 4 h. When injected into either the Arc [F(144,1,008) = 9.8, P < 0.0001] or PVN [F(144,1,008) = 50.5, P < 0.0001], ghrelin elicited an increase in RQ within the first 20–40 min of testing. The potentiation began to decline to near control values by the end of the 4-h test. Although all doses of ghrelin reliably increased RQ, for clarity, only the 30-pmol dose is depicted graphically. No sex differences were observed.

The impact of ghrelin pretreatment on eating elicited by PVN NPY is illustrated in Fig. 5. Significance is represented compared with NPY paired with vehicle. Note that while the vehicle-vehicle condition is not shown, NPY (paired with vehicle) did significantly increase food intake. In both male and female rats, PVN injections of ghrelin potentiated or enhanced eating resulting from NPY treatment [F(4,64) = 78.1, P < 0.001]. In male rats, this occurred at all doses of ghrelin, including low doses of 2.5 and 5 pmol, which we have previously reported to be subthreshold (16). Pilot work in this study also confirmed their ineffectiveness in stimulating eating
or altering RQ when injected alone (data not shown). In female rats, both the 5-pmol and 10-pmol doses were effective in potentiating NPY-elicited eating. Similarly, as shown in Fig. 6, PVN injections of ghrelin enhanced the effect of NPY on RQ in male and female rodents, respectively. That is, NPY alone increased RQ, and this increase was enhanced by ghrelin pretreatment (all doses for both sexes). For clarity of illustration, only the 5-pmol dose of ghrelin is shown graphically, and values are given in 20-min intervals. The potentiation was evident within 40 min of injection and persisted throughout the remainder of the 2-h test session.

**DISCUSSION**

In the present study, ghrelin stimulated eating when injected into the Arc and PVN. This effect was observed in both male and female rats. Additionally, ghrelin injections into these same anatomical sites within the hypothalamus altered energy substrate utilization as reflected in an increase in RQ. The elevation in RQ elicited by Arc and PVN injections of ghrelin, to values near or exceeding 1.0, indicates that the peptide acts locally to promote carbohydrate oxidation and fat storage (7, 33). The diversion of metabolism away from fat oxidation in favor of carbohydrate use and fat synthesis, and its orexigenic action, are in fact consistent with the effects of NPY administration into the PVN (10–12, 15). Indeed, our findings demonstrate that ghrelin enhances the feeding stimulant action of

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Fig. 2. Food intake in male (A) and female (B) rats following microinjection of ghrelin into the paraventricular nucleus (PVN). Values represent mean intakes ± SE measured at 2 h and 4 h. *P < 0.05 compared with vehicle (VEH).

Fig. 3. Mean ± SE respiratory quotient (RQ) following Arc injection of ghrelin (30 pmol) in male and female rats measured over 4 h.

Fig. 4. Mean ± SE RQ after PVN administration of ghrelin (30 pmol) in male and female rats measured over 4 h.

Fig. 5. Food intake mean ± SE over the initial 2 h of the dark cycle following PVN injections of ghrelin and neuropeptide Y (NPY; 50 pmol). *P < 0.05 compared with NPY paired with vehicle.
NPY and potentiates NPY’s effects on RQ, and we did not observe any sex-related differences.

Ghrelin binds to a G-protein coupled receptor, the GHS-R, which has two subtypes, 1a and 1b, and their sequences do not show significant homology with other known receptors (58). The GHS-R 1a is believed to be highly conserved across species, and the human 1a subtype shares 96% identity with the rat 1a receptor (58). Although ghrelin and synthetic GHS bind with high affinity to the GHS1a receptor, this is not the case with the GHS1b, and the functional role of the 1b receptor remains poorly defined (30, 41). Expression for the GHS1a receptor is reported in the hypothalamus and anterior pituitary (30, 56). Recently, a transgenic rat model overexpressing antisense oligonucleotides against the GHS-R 1a was reported to exhibit decreased food intake and lower body fat (57), consistent with ghrelin’s hypothesized positive action on energy balance.

The precise mechanism of action of ghrelin in energy homeostasis remains controversial. Circulating ghrelin is derived largely from the stomach and intestine (18, 21, 48). If ghrelin readily crosses the blood-brain barrier, then peripheral ghrelin might be expected to activate hypothalamic receptors. However, Banks et al. (5) suggest that the quantity of ghrelin transport in the blood to brain direction appears negligible (5). In a recent study, the expression patterns of ghrelin in the hypothalamus were measured (9). Expression of ghrelin was localized to a novel, previously uncharacterized cell group. These neurons are adjacent to the third ventricle and localized between the PVN, Arc, ventromedial hypothalamus, and dorsomedial hypothalamus. Their axonal projections target key hypothalamic circuits producing NPY, AGRP, proopiomelanocortin (POMC) and corticotropin releasing hormone (CRH) in both the Arc and PVN. The identification of this novel distribution of ghrelin provides strong support for a unique role of hypothalamic ghrelin in eating and energy metabolism in addition to its documented role as a peripheral orexigenic peptide. Therefore, at the level of the Arc and PVN, ghrelin may stimulate the release of orexigenic neurotransmitters controlling energy balance and metabolic function. Cowley et al. (9) have also reported that within the hypothalamus, ghrelin binds largely to presynaptic NPY terminals and stimulates the activity of ARC NPY neurons; its actions within the PVN parallel or resemble those of PVN NPY. It seems likely that this close relationship between Arc and PVN NPY underlies the potentiation of NPY’s feeding and RQ effects observed in the present study.

Indeed, our current understanding of the involvement of hypothalamic systems in metabolic regulation indicates that a complex network of neuropeptide and transmitter systems are involved (51, 53, 54, 60). It is also clear that extrahypothalamic mechanisms participate in the process (53, 54). Within the hypothalamus, distinct effects on food intake and energy metabolism have been reported in discrete regions or nuclei. In addition to the effects of NPY described above, some of the other appetite-stimulating neuropeptides include melanin concentrating hormone, hypocretins/orexins, AGRP, CRH and urocortin (6, 19, 44, 49). AGRP is coproduced with NPY in the hypothalamic circuits producing NPY, AGRP, proopiomelanocortin (POMC) and corticotropin releasing hormone (αMSH), and cocaine and amphetamine-related transcript, coproduced in a separate population of Arc neurons (1, 26, 40, 59). Leptin receptors have been localized to the hypothalamus, particularly the Arc nucleus, where they exert primary feedback signaling by influencing the production of appetite-stimulating and inhibiting peptides (20, 22, 39, 69). Interestingly, ghrelin also appears to act on these same leptin-responsive neurons in the rat Arc, and it is now proposed that ghrelin-sensitive circuits are dynamically regulated by leptin (27).

During food deprivation, leptin levels rapidly decline (50), and the production of NPY and AGRP is enhanced, while POMC neuronal activity is suppressed (45–47, 50, 52). At the same time, circulating ghrelin levels increase (2, 64, 65), possibly augmenting the action of leptin by directly activating Arc NPY/AGRP neurons and inhibiting POMC neurons. Thus circulating ghrelin and leptin, although responding to food deprivation in an inverse manner, may regulate hypothalamic peptidergic systems, directed at preventing further energy deficit. Moreover, whereas plasma levels of ghrelin decrease in obesity, leptin levels increase (29, 31, 59, 64, 65), reflecting a physiological adaptation to a state of positive energy balance.

With respect to specific hypothalamic peptidergic circuits, as outlined above, recent attention has focused in large part on Arc neurons producing α-MSH, an anorectic peptide that enhances energy expenditure (42) and their interrelationship with Arc neurons producing both NPY and AGRP (36). AGRP is an endogenous antagonist of α-MSH (36). The interaction of
two distinct populations of neurons is considered significant in the regulation of energy homeostasis. It is proposed that these two major hypothalamic pathways mediate the action of ghrelin on energy balance (41, 55, 63). Ghrelin itself increases AGRP and NPY mRNA levels in the hypothalamus (32). Conversely, the orexigenic action of ghrelin is abolished or reduced by NPY Y1 receptor antagonism, melanocortin agonists, and antisera to both NPY and AGRP (3, 55, 63). Consistent with a direct action on hypothalamic structures, icv ghrelin administration activates c-Fos expression in multiple hypothalamic areas, including the Arc, PVN, dorsomedial nucleus, and lateral hypothalamus (35).

Although relatively few studies have examined the effects of ghrelin on appetite and feeding after direct microinjection into CNS tissue (16, 24, 37, 66, 68), our findings extend current knowledge of the anatomical sites of action within the hypothalamus where ghrelin acts to alter feeding and energy metabolism, and where the peptide interacts with NPY mechanisms. Overall, our data are consistent with the hypothesis that ghrelin alters appetite and energy metabolism, including the promotion of carbohydrate oxidation and the conservation of fat stores, by an action on hypothalamic circuits mediating positive energy balance.

GRANTS
This work is supported by National Institutes of Health Grants MH-070496–01A1 to P. J. Currie and the Undergraduate Biological Sciences Education Program of the Howard Hughes Medical Institute.

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