Stimulation of Appetite by Ghrelin Is Regulated by Leptin Restraint: Peripheral and Central Sites of Action1,2

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ABSTRACT A reciprocal rhythmic pattern of 2 afferent hormonal signals, anorexigenic leptin and orexigenic ghrelin, imparts rhythmicity to the neuropeptide Y (NPY) system, the final common pathway for appetite expression in the hypothalamus. We now show that leptin inhibits both the secretion of gastric ghrelin and the stimulation of feeding by ghrelin. We propose that this dual leptin restraint is the major regulatory arm of the feedback communication between the periphery and the hypothalamus for weight homeostasis, and disruption in the rhythmic communication at any locus in the leptin-ghrelin-NPY feedback loop impels loss of hypothalamic control, leading to abnormal weight gain and obesity. J. Nutr. 135: 1331–1335, 2005.

KEY WORDS: • ghrelin • leptin • rhythms • restraint • obesity

Daily meal patterning is a highly regulated phenomenon. In vertebrates, the intermittent feeding pattern is integrated in the hypothalamus wherein the effector pathways transduce information from metabolic, neural, and hormonal signals and the circadian clock to initiate and terminate a meal (1,2). An interconnected appetite-regulating network (ARN)1 (3) of neuropeptide Y (NPY) and cohorts in the hypothalamic arcuate nucleus-paraventricular nucleus (ARC-PVN) axis is apparently the primary neuroanatomical substrate for elaborating and emitting orexigenic and anorexigenic signals in circadian and ultradian rhythmic patterns (1–4). Various studies show that reciprocal rhythmicities in 2 peripheral hormones, anorexigenic leptin from adipocytes and orexigenic ghrelin from the stomach, are the major afferent signals for the timely activation of the ARN (2,4–6) (Fig. 1). Our concerted efforts to delineate feedback communication between the periphery and ARN for maintenance of energy homeostasis on a daily basis has provided new insights at several fronts. These include: 1) existence of a temporal causal relation between rhythmic NPY secretion in the ARC-PVN axis and rhythmic afferent hormonal feedback signals (2,5–8), 2) evidence that derangement in onset, periodicity, duration, or magnitude of afferent feedback signaling imposes a corresponding abnormality in periodic NPY discharge that precedes excessive energy intake and fat accretion (3,4,8), 3) identification of leptin as the primary signal that concurrently augments nonshivering thermogenesis and restrains the orexigenic effects of ghrelin at central and peripheral targets (9–13).

These major findings, derived from measurement of dynamic minute-to-minute fluctuations of various signals, correction of central deficits by gene transfer in vivo, and temporal sequencing of molecular and cellular events engaged in initiation and termination of daily meal patterning are summarized in this article.

Ghrelin: a peripheral and central orexigen

Among a spectrum of peripheral afferent hormonal signals examined so far, ghrelin is currently the only known hormone to readily stimulate feeding and promote adiposity after peripheral administration (1,2,14,15). In addition, the ghrelin produced by neurons in the subparaventricular zone of the hypothalamus is also believed to be orexigenic within the ARN (16). Several lines of evidence suggest that ghrelin may serve as one of the physiologically relevant signals in stimula-
Effects of ghrelin; episodic fluctuations in energy reserves. Ghrelin is normally secreted in an episodic fashion (5) (Fig. 2). A reciprocal relation between circulating ghrelin and leptin is also seen normally on a daily basis in rats. Premeal ghrelin hypersecretion at the onset of dark-phase ingestive behavior and preceding the time of food availability in a scheduled feeding paradigm is coincident with low circulating levels of leptin (19,23). On the other hand, a gradual rise in leptin hypersecretion precedes the postprandial decline in ghrelin secretion (19,22,23).

We have observed a reciprocal relation in ghrelin and leptin secretion in 2 additional experimental paradigms aimed at increasing or decreasing adiposity. In outbred Sprague-Dawley rats consuming a diet high in energy, the obesity-prone (OP) group of rats displayed accelerated weight gain in a time-related fashion, whereas the obesity-resistant (OR) group maintained weight in the range of control rats consuming normal rat diet (24,25). Analysis of the rhythmic leptin and ghrelin secretion in these rats showed that only the OP rats rapidly developed hyperleptinemia characterized by high-amplitude leptin episodic discharge (24), accompanied by drastically suppressed ghrelin episodes. Leptin and ghrelin secretion patterns were unchanged in OR and control rats. These findings raised the possibility that hyperleptinemia may suppress ghrelin secretion and this may account for the reported suppressed ghrelin secretion in obese patients and rats (12,13,18).

There is also an opposing relation, ghrelin hypersecretion in conjunction with leptinopenia in another experimental model. A nonpathogenic and replicative deficient vector, recombinant adeno-associated virus encoding the leptin gene (rAAV-lep), injected either intracerebroventricularly or microinjected into discrete hypothalamic sites of normal or diet-induced obese rats and mice, suppressed weight gain and adiposity and markedly suppressed circulating leptin levels for over 1 y duration of the experiments (11,13,25–29). Quite unexpectedly, we uniformly observed ghrelin hypersecretion accompanying the prolonged and severe leptinopenia and loss of adiposity in these rats and mice. Remarkably, despite markedly increased circulating ghrelin level in these rAAV-lep-treated rats and mice, food intake was suppressed. Evidently, a central restraint on the appetite-stimulating effects of ghrelin in these leptin transgene–expressing rats and mice was in effect.

Leptinopenia concomitant with increased episodic ghrelin secretion in food-deprived and rAAV-lep treated mice on the one hand and hyperleptinemia associated with diminished intermittent ghrelin output in obese mice on the other, together with the reciprocal relation between these 2 hormones pre- and postprandially, led us to postulate that leptin may exert a tonic restraint on ghrelin secretion from the stomach (12,13). Indeed, in support of this formulation a single systemic leptin injection to rAAV-lep-treated leptinopenic and ghrelin hypersекreting wild-type and ob/ob mice rapidly suppressed ghrelin secretion (12,13) (Fig. 4). In support of this in vivo evidence, leptin also suppresses ghrelin release from isolated stomach in vitro (30).

**FIGURE 1** The dynamics in the feedback circuitry involved in integration of energy intake and expenditure are shown. NPY, GABA, and Agrp produced and released in the ARC-PVN axis regulate the daily episodic pattern of feeding. The 2 rhythmic patterns of NPY release (ultradian and circadian) are directed by 2 functionally opposing rhythmic afferent hormonal signals—leptin from adipocytes and ghrelin from the stomach. Also depicted is the rhythmic feedback relation in the adipocyte-stomach-pancreas axis. \( \Rightarrow \) restraint on orexigenic effects of ghrelin; (+) = stimulatory, (–) = inhibitory, (±) and ? = unresolved. For details see text. Reprinted with permission from ref. (2).
Thus, the endocrine cells—adipocytes and oxyntic cells—are endowed with cellular mechanisms to discharge leptin and ghrelin intermittently at a basal rate, and each is vulnerable to modulation by converging metabolic, hormonal, and neural information (2,4–10). Further, an exquisite temporal relation exists between ghrelin and leptin in the periphery, and the restraining influence of leptin on gastric ghrelin output governs the strength of their reciprocal feedback signaling to the hypothalamic ARN (2,4,12,13,24).

Ghrelin and leptin interplay in the ARC-PVN axis for energy homeostasis

The current view holds that gastric ghrelin crosses the blood brain barrier and, in concert with ghrelin produced locally in the hypothalamus, engages the network of NPY and cohorts in the ARC-PVN axis to evoke the appetitive drive (2,31). NPY neurons coexpress ghrelin receptors and the orexigen, agouti-related peptide (AgrP) and γ-aminobutyric acid (GABA) (1–3,8). On the basis of the cumulative evidence that leptinemia after fasting or preceding the onset of a meal is contemporaneous with enhanced pulsatile ghrelin secretion in the periphery, and the restraining influence of leptin on gastric ghrelin output governs the strength of their reciprocal feedback signaling to the hypothalamic ARN (2,4,12,13,24).

A central effect of leptin is to restrain food intake by suppressing NPY synthesis, release, and action in the ARC-PVN axis (1–4). This tonic restraint is mediated through activation of the biologically relevant long form of leptin receptors expressed by NPY neurons in the ARC and by NPY targets sites coexpressing Y1/Y5 receptors in the mPVN (34–37). Is the dynamic site-specific interplay of leptin and ghrelin in the ARC-PVN axis responsible for episodic appetitive drive? Contrary to expectations, we observed that leptinopenic rAAV-lep-treated rats and mice ate less despite markedly elevated blood ghrelin levels (11–13,25,28,29). Because NPY expression in the ARC was drastically suppressed by the locally expressed leptin in these rats and mice (11,26–28), this implied that even a robust peripheral ghrelin signal was incapable of countering the leptin restraint on NPYergic signaling. Indeed, this was the case because exogenous ghrelin that readily stimulated feeding in a dose-dependent manner in
control mice was ineffective in mice expressing leptin locally in the hypothalamus (12,13) (Fig. 5). Evidently leptin counteracts the ghrelin-induced activation of NPYergic signaling at the level of the NPY ARC-PVN axis (Fig. 1).

**CONCLUSIONS**

These studies demonstrate that a tonic dual restraint by leptin on gastric ghrelin secretion peripherally and on activation by ghrelin of the orexigenic network of NPY and cohorts centrally is crucial in the dynamic operation of the leptin-ghrelin-NPY feedback loop (Fig. 1). We envision that the daily ebb and flow in the tonic restraint imposed by leptin on peripheral and hypothalamic orexigenic pathways not only generates the daily pattern of energy intake, but also propagates the circadian and, possibly, the ultradian rhythmic patterns of ghrelin and NPY secretion (2–4,39). Further, it is likely that consumption of high-energy diets disrupts 1 or more

**FIGURE 3** Representative profiles of pulsatile leptin pattern in individual AD (solid circles) and food-deprived rats (empty circles). The mean number of pulses, amplitudes, and interpulse intervals in the 2 groups are also depicted. *P < 0.05 vs. AD group. Reprinted with permission from (5).

**FIGURE 4** The effect of an i.p. leptin injection on plasma ghrelin, in wild-type (wt, A) and ob/ob (B) mice on d 35–42 post-icv treatment with either rAAV-GFP (green fluorescent protein control) or rAAV-lep. Note the high initial ghrelin levels in wt and ob/ob mice injected with rAAV-lep. *P < 0.05 vs. initial values, #P < 0.05 vs. control (saline) group. Reprinted with permission from ref. (13).

**FIGURE 5** The effect of an i.p. ghrelin injection on food intake in untreated and wt mice at 37–49 d post-icv treatment with either rAAV-GFP or rAAV-lep. *P < 0.05 vs. saline group. Reprinted with permission from ref. (13).
locus in this feedback communication to culminate in storage of excess energy as fat (1–4,8,24,39). Consequently, to curb the pandemic of obesity, newer interventional therapies should aim at reinstating the physiological homeostatic reciprocal relation between leptin and ghrelin on a moment-to-moment basis.

LITERATURE CITED


