Ghrelin—Satisfying a Hunger for the Mechanism

Ghrelin was first described in 1999 by Kojima et al. (1); it was identified as the long-awaited endogenous ligand to the GH secretagogue receptor (GHS R) and a peripheral metabolic signal informing the brain about stomach nutrient load. Ghrelin quickly became the focus of intense investigation in many laboratories and was the subject of more than 600 publications by early 2004. Because of the potential importance of ghrelin in the regulation of feeding, it was originally promoted as a target for the treatment of obesity; however, it may be more immediately useful in the treatment of wasting syndromes, driving increased food intake and GH release. Regardless, a hormone released by an empty stomach presents a novel drug development opportunity. Can blocking ghrelin suppress hunger and decrease body weight? It is well recognized that hypothalamic circuits, especially arcuate circuits, inform the brain about the energy status of the body. Although there is strong evidence supporting a hypothalamic mode of action, there is also evidence that ghrelin may work via the hindbrain. Therefore, there is still debate about the mechanism of action as well the circuitry by which ghrelin modifies feeding. The paper by Chen et al. (2) in this issue of *Endocrinology* is a timely report providing compelling evidence that peripheral ghrelin acts through hypothalamic neuropeptide Y (NPY)/Agouti-related peptide (AgRP) and proopiomelanocortin (POMC) neurons to stimulate feeding, hopefully ending the debate about the main site of action of ghrelin.

**Does ghrelin induce feeding through NPY/AgRP neurons?**

The key findings in this paper (2) are that deletion of NPY attenuates the ghrelin-induced feeding, whereas responses in AgRKO knockout mice are normal. However, NPY/AgRP double mutants have no response to ghrelin. This data accords well with our data (3) and others (4, 5) showing that ghrelin increases the activity of arcuate nucleus NPY neurons (which we know coexpress AgRP). Furthermore, intraarcuate injection of ghrelin stimulates feeding (6), and NPY neurons show increased expression of c-fos in response to peripheral ghrelin or GHS receptor agonists (7–12). Importantly, this paper shows not only that ghrelin works through NPY/AgRP neurons, but also that ghrelin only works if NPY and AgRP are present. Thus, Chen et al. (2) identify these neurons as the sole mediators of ghrelin-stimulated feeding.

These studies are the most complete molecular genetics studies to address the mechanism by which ghrelin stimulates feeding in animals. A molecular genetic approach is necessary to answer these questions because of the unique biology of the system; although we can pharmacologically dissect the role of NPY in the ghrelin response, it is difficult to imagine a robust alternative approach to block the actions of the melanocortin 4 receptor (MC4 R) antagonist AgRP. Indeed, genetic deletion is probably the only convincing way to understand the role of AgRP in the melanocortin circuit. By melanocortin circuit, we are referring to the NPY/AgRP neurons as well as POMC neurons. We refer to the main output of the arcuate nucleus as being a melanocortin circuit because both arms of this circuit exert effects upon melanocortin receptors.

**Peptides matter**

These studies show that ghrelin has an absolute requirement for NPY pathways and that full stimulation of feeding activity requires both NPY and AgRP. They furthermore show that MC4 R-deficient mice have an attenuated response to ghrelin or ghrelin mimetics, in contrast to AgRP-deficient mice, again pointing to a crucial role for AgRP/α-MSH coordinate actions at the MC4 R. It would be interesting to see whether the feeding response was completely absent in mice that were deficient in MC4 R and NPY, because it would be assumed that there would be no orexigenic drive in the melanocortin circuits of such mice.

In many ways, the results reported in the study by Chen et al. (2) are surprising in that simply knocking out the neuropeptides NPY/AgRP eliminated the feeding response to peripheral ghrelin. These findings are unexpected because it is well recognized that neurotransmitters such as γ-aminobutyric acid (GABA) and glutamate are essential to the regulation of the arcuate circuit. van den Pol (13) highlighted this thinking in a recent review. His central thesis might be paraphrased with the statement “Transmitters matter,” and it has been supported by extensive electrophysiology studies. So, although Chen et al. (2) establish that ghrelin works selectively through NPY/AgRP neurons within the arcuate nucleus, it is often forgotten that these are GABAergic neurons that corelease NPY/AgRP. Does ghrelin not use GABA release from these neurons? Does it preferentially release NPY/AgRP? As it turns out, this may indeed be the case. Spanswick and co-workers (4) recently demonstrated that ghrelin increases the firing rate of NPY/AgRP neurons by inducing a bursting pattern at the peak of rhythmically oscillating membrane potential, which favors peptide release. Therefore, changes in GABAergic transmission by ghrelin, reported by ourselves (3), may be secondary to the release of NPY/AgRP. Perhaps we need to revisit the summary of van den Pol’s review, with the addendum “Transmitters matter, sometimes.”

The role of neurotransmitters in the neuronal response to metabolic signals is further complicated by recent work showing that POMC neurons are also GABAergic (14). As soon as it was clearly understood that NPY/AgRP neurons were GABAergic, many of us had simplistically expected

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**Abbreviations**: AgRP, Agouti-related peptide; GABA, γ-aminobutyric acid; GHS R, GH secretagogue receptor; MC4 R, melanocortin 4 receptor; NPY, neuropeptide Y; POMC, proopiomelanocortin.
that POMC neurons would secrete glutamate as a neurotransmitter. This would fit nicely with the developing model that POMC and NPY/AgRP neurons acted antagonistically to one another and were regulated in reciprocal ways. Thus, we expected that the competition would also occur at the level of neurotransmitters. Not so simple, it seems.

This effect of ghrelin is unique in other ways, too. It has been demonstrated by several laboratories that although leptin directly affects this circuit, it does so by a different mode of action. Leptin directly increases the activity of POMC neurons (15), causing more frequent action potentials, and decreases the activity of NPY neurons, but it does not appear to change the mode of activity of these neurons. Furthermore, leptin and ghrelin have different effects upon synaptic plasticity in arcuate nucleus neurons. In a recent paper, Pinto et al. (16) demonstrate that leptin increases the excitatory tone and reduces the inhibitory tone onto POMC neurons and has the opposite effects on NPY neurons; it increases inhibitory tone and decreases excitatory tone onto NPY neurons. In contrast, ghrelin does not alter synaptic inputs onto NPY neurons but does increase the inhibitory tone onto POMC neurons.

One of the other surprising findings of this study is that knocking out NPY alone significantly decreases ghrelin-stimulated feeding, as previously reported using a GHS R agonist (17), but knocking out AgRP alone has no effect. There is no solid evidence that ghrelin can preferentially regulate NPY vs. AgRP, but rather ghrelin appears to increase the expression of both NPY and AgRP (9, 18). Do these data suggest that NPY is the primary signal in these neurons? This makes physiological sense because NPY works at two sites, locally within the arcuate nucleus to inhibit POMC neuronal activity and at afferent-terminal sites, i.e., paraventricular nucleus of the hypothalamus, dorsomedial nucleus of the hypothalamus, and the lateral hypothalamic area (Fig. 1). In contrast, AgRP likely works only at these terminal afferent sites to block melanocortin tone. Therefore, in the NPY knockout mouse, ghrelin would not be able to generate the orexigenic drive at the afferent-terminal sites and would not inhibit POMC neuronal activity, whereas in the AgRP knock-out mouse, ghrelin is able to activate the NPY orexigenic drive and suppress POMC activity through the local NPY circuit in the arcuate nucleus. Therefore, AgRP-antagonizing melanocortin tone at the afferent target sites may not be necessary. The double knockout mouse simply has no NPY orexigenic drive, no local modification of POMC activity, and no inhibition of melanocortin tone at afferent target sites. A major caveat is that the double knockouts themselves have no abnormal basal feeding activity. Why is this? Likely leptin is able to keep the system in check. As mentioned, leptin can generate GABAergic transmission from these NPY/AgRP neurons, even in the absence of NPY/AgRP corelease, and leptin can directly modify POMC neurons.

It is also interesting to note that the phenotype of ghrelin-deficient mice is at best subtle (19,20). With the new evidence provided by Chen et al. (2), the lack of phenotype in the ghrelin knockout mouse may not be surprising, simply because ghrelin works linearly through modulation of NPY/AgRP neurons and the NPY/AgRP double knockouts don’t have a significant phenotype under basal conditions.

There is no compelling evidence that mutations in ghrelin or GHS R contribute to human obesity, and this is consistent with the subtle phenotype of ghrelin-deficient mice. However, normal-weight and obese humans may not be the best populations to look at for mutations in the ghrelin-signaling system; perhaps groups that have abnormal responses to wasting or cachexia (either resistance or susceptibility) may prove enriched for ghrelin system mutations. The lack of population genetic evidence for ghrelin playing an important role in energy homeostasis is in contrast to the growing body of evidence that ghrelin levels predict relapse after bariatric surgery. It is worth noting that Prader-Willi patients, who have uncontrollable feeding behavior, have elevated ghrelin (21, 22).

Implications

The findings outlined here are difficult to reconcile with the work of Date et al. (23) showing that ghrelin activates vagal nerves and that vagotomized animals have no response to peripheral ghrelin but do respond to intracerebroventricular ghrelin. The reasons for the profound difference are not clear. It might be argued that peripheral ghrelin acts by effects on gastric vagal afferents and that these nerves eventually alter the activity of hypothalamic NPY/AgRP circuits via a hindbrain relay. The presence of GHS R in the arcuate NPY neurons suggests that ghrelin also acts directly on the NPY/AgRP neurons. However, the ghrelin that may act directly on NPY neurons in the arcuate nucleus may not be derived from the stomach. We have recently shown that ghrelin is produced in the brain and fibers that express ghrelin make synapses onto NPY neurons in the arcuate nucleus (3). Thus, the primary questions become “does ghrelin signaling reach the arcuate nucleus via a hindbrain relay or via transport across the blood brain barrier?” and “does centrally derived ghrelin have effects upon arcuate nucleus neurons?” The first question might be addressed by NPY/AgRP neuron-specific deletion of GHS R or by determining the extent of c-fos expression induced in NPY/AgRP neurons by peripheral ghrelin to decerebrate animals. The
last question will be a challenge to address because of the modest phenotype of the ghrelin-deficient mice, which will make it very complex to determine the difference between central and global ghrelin deficit. Whatever the circuit, the data presented by Chen et al. (2) point to the NPY/AgRP neurons in the arcuate as being the key link in this hunger mechanism.

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References


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