All roads lead to presynaptic calcium channel inhibition by the ghrelin receptor: Separate agonist-dependent and -independent signaling pathways

Norbert Weiss¹ and Gerald W. Zamponi²

¹Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., 199 00 Prague 9, Czech Republic
²Department of Physiology and Pharmacology, Cumming School of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada

In the late 1970s, Dunlap and Fischbach (1978) reported that synaptic activity in sensory neurons is modulated by neurotransmitters, and they subsequently attributed this phenomenon to the inhibition of voltage-gated Ca²⁺ channels (VGCCs) (Dunlap and Fischbach, 1981). Their seminal studies laid the groundwork for three decades of research that revealed how G protein–coupled receptors (GPCRs) and G protein signaling play an essential role in the regulation of Ca²⁺ entry at presynaptic nerve endings and the fine-tuning of neurotransmitter release. Inhibition of VGCCs by GPCRs is crucial for regulating the excitability and responsiveness of nerve cells and neuronal networks under typical physiological conditions. This inhibition is also exploited for therapeutic purposes, as underscored by the treatment of conditions. This inhibition is typi-
cally fast (i.e., develops within tens of milliseconds); and most frequently reported for the Gq/o-coupled receptors, although Gs- and Goq-coupled receptors can also initiate this pathway. Gbγ-mediated inhibition is less pronounced at depolarized membrane potentials, and can be experimentally relieved by short, highly depolarizing voltage steps, giving rise to the term “voltage-depen-
dent” regulation. In contrast, Go-mediated inhibition, which is less well characterized, typically involves one of many downstream diffusible messengers and signaling pathways involving phosphorylation and lipid metabolism. Go-mediated inhibition is comparatively slow and develops within tens of seconds, and is in essence “voltage independent” (i.e., it is not relieved by membrane depolarization steps). An additional level of regulation arises from the direct biochemical association of the Ca²⁺ channel with the GPCR to not only control the traf-
ficking of the channel to the cell surface but also give rise to an agonist-mediated cointernalization of the channel–receptor complex, thus regulating channel density in the plasma membrane (Zamponi, 2015).

Association of channels with receptors can also support a tonic, voltage-dependent inhibition of the channel in the absence of receptor agonist, which is believed to arise from constitutive receptor activity and its associated activation of the Gbγ pathway. Increasing evidence supports a vital role of agonist-independent constitutive activity of GPCRs in the control of neuronal network activity, and to date, more than 60 GPCRs have been reported to exhibit constitutive activity (Meye et al., 2014). A new study by the group of Jesica Raingo, re-
ported in this issue (López Soto et al.), sheds light on a previously unidentified regulatory pathway of neuronal VGCCs that is mediated by the growth hormone secre-
tagogue receptor type 1a (GHSR1a). GHSR1a is an un-
usual GPCR in that, although it is broadly expressed in the central nervous system, its natural agonist ghrelin is generally undetectable other than in trace amounts in the hypothalamus. However, GHSR1a exhibits among the highest constitutive activities of members of the GPCR family, and agonist-independent constitutive activity of GHSR1a has been proposed to modulate neuronal...
activity, especially in the hypothalamus, where it controls hunger by modulating the hypothalamo–pituitary–adrenal axis (Wren et al., 2000). The implication of GHSR1a in appetite and energy metabolism is best exemplified by the occurrence of human mutations that cause a loss of GHSR1a constitutive activity, leading to short stature caused by growth hormone deficiency (Pantel et al., 2006). However, the signaling pathways and downstream effectors by which agonist-independent constitutive activity of GHSR1a regulates neuronal networks had remained incompletely understood.

López Soto et al. (2015) used a combination of electrophysiological recordings combined with genetic and pharmacological manipulations of GHSR1a activity/ expression to test the hypothesis that GHSR1a may control neuronal activity by modulating presynaptic VGCCs and neurotransmitter release. The authors showed that Ca,2.1 and Ca,2.2 channels expressed in HEK cells are sensitive to modulation by GHSR1a. Notably, they demonstrated that ghrelin induces a fast, voltage-dependent, and agonist-dependent inhibition of the Ca,2 currents that is mediated by the direct inhibition of the channel by G protein βγ dimer and involves Gαq signaling. In addition, they demonstrated that constitutive activity of GHSR1a inhibits both Ca,2.1 and Ca,2.2 channels via an agonist-independent mechanism that depends on the expression level of the receptor, requires Gαi/o proteins, and is associated with a reduced Ca,2 channel density at the cell surface. Agonist-independent inhibition was not reproduced with a GHSR1a mutant that lacks constitutive activity, indicating that this regulatory pathway is not mediated by the receptor itself but requires activation of downstream signaling molecules.

To further analyze whether GHSR1a modulates VGCCs in native cells, López Soto et al. (2015) performed electrophysiological recording on primary hypothalamic neurons from transgenic GHSR-eGFP mice. Consistent with their initial observation, the application of ghrelin inhibited Ca,2.1 and Ca,2.2 channels in neurons expressing GHSR. In addition, GHSR-positive neurons displayed decreased Ca,2 current density, indicating that GHSR1a-induced agonist-independent inhibition occurs in a physiologically relevant setting. To confirm that ghrelin-independent inhibition is mediated by constitutive activity of GHSR, the authors transfected hypothalamic neurons with a GHSR1a mutant lacking constitutive activity, and found that Ca,2 currents were increased compared with neurons expressing the wild-type receptor. Finally, López Soto et al. assessed whether GHSR-mediated inhibition of Ca,2 channels affects neurotransmitter release in hypothalamic GABAergic neurons. This is particularly relevant, as it has been reported that γ-aminobutyric acid (GABA) release is essential for regulating food intake (Wu et al., 2009), and that GHSR1a activity decreases inhibitory input of hypothalamic networks (Cabral et al., 2012). To make such studies possible, the authors used hypothalamic tissue explants of the arcuate nucleus (ARC) from fasted mice in which expression of GHSR1a is increased. Consistent with the hypothesis that increased GHSR1a expression inhibits presynaptic VGCCs and thus neurotransmitter release, these authors found that GABA release induced by high potassium depolarization is decreased in explants from fasted mice compared with explants from ad libitum–fed mice. Altered GABA release was further documented by recording of inhibitory postsynaptic currents (IPSCs) in hypothalamic neurons. The amplitude of IPSCs recorded from cultures expressing GHSR1a was significantly reduced compared with those in cultures expressing the mutant GHSR1. This effect was fully attributed to the alteration of Ca,2-dependent presynaptic GABA release.

The novel and important findings of López Soto et al. (2015) provide compelling evidence for a role of GHSR1a in the control of the hypothalamic network, especially in the modulation of GABAergic activity in the ARC nucleus. Ghrelin-mediated hunger is believed to rely on the modulation of neuronal circuits within the ARC nucleus, where two different subpopulations of neurons are involved. The pro-opiomelanocortin (POMC) /cocaine- and amphetamine-regulated transcript (CART) neurons are involved in the generation of an orexigenic signal. In contrast, agouti-related peptide (AgRP)/neuropeptide Y (NPY)/GABA neurons are involved in the generation of an orexigenic signal. Both POMC/CART and AgRP/NPY/GABA neurons project to anorexigenic neurons in the paraventricular nucleus (PVN), and to orexigenic neurons located in the lateral (LHA) and perifornical (PFA) regions of the posterior hypothalamus, which in turn project to the nucleus of the solitary tract (NTS) to modulate food intake (Fig. 1). Ghrelin is believed to bind to and stimulate AgRP/NPY/GABA neurons expressing GHSR1a, which has two main effects: (1) inhibition of POMC/CART neuron activity through the inhibitory GABAergic input from activated AgRP/NPY/GABA neurons (which in turn suppresses the anorexigenic tone in the PVN and relieves the inhibitory input of orexigenic neurons from POMC/CART neurons), and (2) a direct stimulation of the orexigenic pathway by activated AgRP/NPY/GABA neurons. As a consequence, an orexigenic signal is sent to the NTS, which triggers feeding behavior.

The exact downstream intracellular mechanisms by which ghrelin stimulates AgRP/NPY/GABA neurons are not fully understood, but it was proposed that the binding of ghrelin to GHSR1a induces an increase of intracellular Ca,2 concentration mediated by a PKA-dependent potentiation of Ca,2.2 channels, and increased neurotransmitter release (Kohno et al., 2003). In contrast, the findings of López Soto et al. (2015) support an inhibition of neuronal Ca,2 channels and GABA release in an agonist-dependent and -independent manner. A physiological consequence therefore is a
suppression of the inhibitory GABAergic input of POMC/CART neurons, which in turn is expected to stimulate the anorexigenic pathway. Thus, this contrasts with the established role of ghrelin in stimulating appetite. The work of López Soto et al. highlights the complexities of the ghrelin system in the control of appetite, and may help us understand the apparent discrepancy between results showing reduced weight in diet-induced obese mice treated with GHSR1a antagonists, whereas other studies reported a gain weight (Patterson et al., 2011).

In addition to providing essential information on the regulation of the hypothalamic network by the ghrelin–GHSR1a system, López Soto et al. also shed light on a new GPCR signaling pathway through which GPCRs control the density of presynaptic Ca\textsuperscript{2+} channels at the cell surface, thus providing for an additional level of control of synaptic activity. Control of channel surface density by GPCRs was reported previously for Ca\textsubscript{2.2} channels complexed with nociceptin (ORL1) and dopamine (D1 and D2) receptors (Zamponi, 2015). This regulation relies on the direct interaction between the channel and the receptor, leading to channel–receptor co-trafficking to the plasma membrane and agonist-dependent cointernalization (Altier et al., 2006). An association of GHSR1a with D2 receptors has been reported in thalamic neurons, which in turn modulated dopamine signaling and appetite (Kern et al., 2012). Hence, it is possible that a similar association with neuronal Ca\textsuperscript{2+} channels exists and contributes to the control of channel surface expression by GHSR1a. However, the observation that a GHSR1a lacking constitutive activity failed to modulate channel surface density suggests that G protein signaling also plays a crucial role in this control. There is evidence in the literature that heterotrimeric G proteins, especially Go\textsubscript{i} signaling, contribute to

Figure 1. Ghrelin receptor signaling in the hypothalamus. (A) Hypothalamic circuits involved in ghrelin-mediated feeding behavior. Two distinct neuronal populations in the ARC represented by ArGP/NPY/GABA neurons and POMC/CART neurons are considered first-order sensory neurons in the control of food intake. They project to anorexigenic neurons in the PVN and orexigenic neurons in the PFA and LHA, which in turn project to the NTS to control feeding behavior. Upon stimulation of ArGP/NPY/GABA neuron activity by ghrelin, POMC/CART neurons are inhibited through GABAergic input from activated ArGP/NPY/GABA neurons, which in turn suppress the anorexigenic tone in the PVN. This inhibition may be balanced by the inhibition of Ca\textsubscript{2.1} and Ca\textsubscript{2.2} channels on the ArGP/NPY/GABA neurons by presynaptic GHSR1a (dotted circle). This in turn inhibits GABA release and suppresses inhibitory GABAergic input of POMC/CART neurons. The orexigenic and anorexigenic pathways are outlined in green (plus symbols) and red (minus symbols), respectively. (B) GHSR1a-mediated inhibition of presynaptic Ca\textsubscript{2.1} and Ca\textsubscript{2.2} channels occurs via two distinct mechanisms: (1) an agonist-dependent inhibition that involves the activation of Go\textsubscript{q} coupled receptors and a direct channel inhibition by G\textsubscript{q}βγ dimer; and (2) an agonist-independent inhibition mediated by constitutive GHSR1a activity and Go\textsubscript{i}/o signaling that not only supports G\textsubscript{q}γ-mediated inhibition but also decreases channel density in the plasma membrane, possibly by stimulating the internalization of channel–receptor complexes or by suppressing channel trafficking to the cell surface.
the control of membrane trafficking and regulate exocytosis from intracellular organelles (Stow et al., 1991; Aridor et al., 1993). In addition, G proteins of the Goi family have been reported to control the trafficking of aquaporin 2 channels in kidney epithelial cells (Valenti et al., 1998), and also the trafficking and distribution of connexin 43 hemichannels in Novikoff hepatoma cells (Lampe et al., 2001). In this last study, the inhibition of G proteins with pertussis toxin resembled the effects of the trafficking inhibitor brefeldin A, supporting a key role of Goi signaling in the transport of ion channels to the plasma membrane. All of these aspects of G protein signaling in intracellular trafficking are certainly of direct relevance for the observation of López Soto et al. (2015) on the role of GHSR1a in controlling Ca\textsuperscript{2+} channel plasma membrane density. Further investigation is warranted to identify the precise molecular details of this signaling mechanism.

Overall, the finding of López Soto et al. provide novel insights into the physiology of the hypothalamus, and establish the ghrelin-GHSR1a signaling pathway as a key regulator of neuronal Ca\textsuperscript{2+} channels and synaptic activity in the control of the hypothalamic networks.

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