COMMENTARY

Orexin neurons use endocannabinoids to break obesity-induced inhibition

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Obesity is a pressing health problem affecting more than one-third of adults in the United States and Europe. Besides their increased risk to develop cardiovascular disease and type 2 diabetes, metabolic disturbances in overweight individuals affect sleeping behavior, promoting arousal and feeding (1). Task-dependent recruitment of diverse neuropeptidergic neurons in hypothalamic nuclei orchestrates distinct endocrine functions particularly relevant to maintain energy homeostasis (2). The interplay of neuropeptide Y (NPY)/agouti-related peptide and α-melanocyte–stimulating hormone (αMSH) neurons of the arcuate nucleus with orexin (hypocretin)-containing neurons in the lateral hypothalamus is central to the regulation of food intake and arousal. A key attribute of these hypothalamic circuits is their remarkable ability to undergo "synaptic rewiring" to maintain the body's energy homeostasis (3, 4). In PNAS, Cristino et al. (5) provide fresh understanding of the molecular regulation of synaptic plasticity by showing how endocannabinoids control inhibitory synapses newly recruited to orexinergic neurons in obesity.

Orexin-containing neurons (up to ∼70,000 in human) (6–8) are mostly located in the lateral hypothalamic nucleus, and are thought to contribute to regulating food intake, wakefulness, and arousal (3, 9). Orexin-containing cells are innervated by NPY and αMSH-containing arcuate neurons (Fig. 1*A*). Neuropeptides typically coexist with fast neurotransmitters (2): NPY neurons contain the inhibitory neurotransmitter GABA, and αMSH can colocalize with excitatory glutamate (10). NPY itself is an orexinergic neuropeptide robustly inducing food intake (11, 12). In contrast, αMSH is anorexigenic, decreasing feeding (13). GABAergic and glutamatergic synaptic inputs to these arcuate neurons are prone to remodeling upon genetic or dietary manipulation of leptin (4), the adipocyte-derived satiety hormone, which critically impacts energy homeostasis and body weight (14). Nevertheless, a lack of consensus exists as to the neurophysiological requirements of neuropeptide and primary fast neurotransmitter corelease from hypothalamic neurons, and their endocrine action.

Excitatory synapses onto orexin-containing neurons vastly outnumber inhibitory terminals (3). The number of these excitatory inputs increases upon food deprivation (3). However, synapse remodeling in the lateral hypothalamus in obesity remains unknown. Using a multiparametric approach encompassing systems neuroanatomy, neurophysiology, molecular pharmacology, and mouse genetics, Cristino et al. (5) identify an excitatory-to-inhibitory switch of synapses impacting orexin-containing neurons in genetically leptin deficient *ob/ob* mice (11). Here, a substantial subset of new inhibitory terminals from NPY-containing neurons replaced excitatory inputs formed by αMSH cells (Fig. 1 *B* and B_1). Significantly, high-fat diet, evoking leptin resistance in arcuate, but not lateral hypothalamic neurons, replicated circuit remodeling. This synaptology was unequivocally driven by the lack of leptin because leptin signaling through the mammalian target of rapamycin rapidly normalized synapse composition.

Orexin, like NPY, is up-regulated upon decreased leptin availability (15). The formation of new inhibitory synapses terminating on orexin-containing neurons in *ob/ob* mice, which lack leptin, would suggest increased synaptic inhibition, decreasing the excitability and perhaps even reducing neuropeptide release from orexin-containing neurons. However, this is clearly not the case because orexin expression, axonal transport, and accumulation in terminals increased in *ob/ob* mice.

Early work by Di Marzo et al. revealed increased concentrations of hypothalamic endocannabinoids in *ob/ob* mice (16). Endocannabinoids, particularly 2-arachidonoyl glycerol (2-AG), are produced upon neuronal activity and released from subsynaptic dendrites to presynaptically reduce

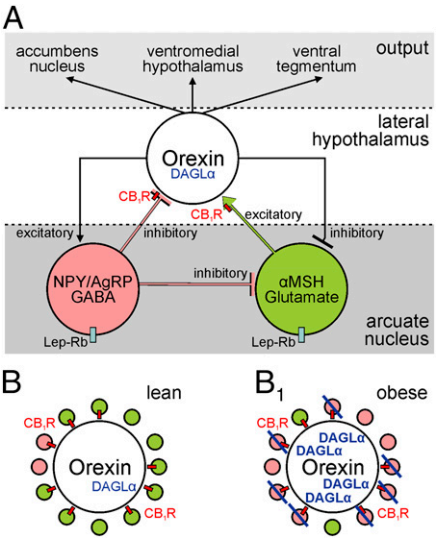

Fig. 1. (*A*) Hierarchical depiction of synaptic connections allowing information flow among neuropeptidergic neurons in the arcuate mucleus and lateral hypothalamus. Lep-Rb denotes leptin receptor expression by neurons in the arcuate nucleus. (*B*) Orexin-containing neurons receive predominantly excitatory synaptic inputs in lean mice. (pink: inhibitory/GABA synapse; green: excitatory/glutamate synapse; red rectangles pinpoint CB_1 cannabinoid receptors). (B_1) In obesity, orexin-containing neurons up-regulate DAGLα to block surplus inhibition by retrograde endocannabinoid signals.

neurotransmitter release (17). Thus, and by adopting a "retrograde" mode of action, endocannabinoid signaling is a form of feedback control of synaptic neurotransmission. 2-AG is thought to be primarily produced by *sn*-1-diacylglycerol lipase α (DAGLα) in the adult brain (18). Cristino et al. (5) exploit this knowledge to demonstrate that the synaptic rewiring of orexin neurons in *ob/ob* mice coincides with their increased DAGLα expression. Because 70% of all synaptic inputs to orexin neurons, including many NPY- and αMSH-containing afferents, contain presynaptic CB_1 cannabinoid receptors (CB_1Rs), Cristino et al. hypothesize that orexin neurons in obese mice can successfully eliminate surplus inhibition by using

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2-AG as retrograde messenger. The authors address the functional significance of synapse reorganization by showing that somatic depolarization of orexin-containing neurons, a means to induce endocannabinoid release (17), in *ob/ob* mice suppressed presynaptic GABA release. The lack of retrograde signaling at inhibitory synapses converging onto orexin-containing neurons in lean mice highlights the network-level significance of the glutamate-to-GABA switch.

Mice lacking monoacylglycerol lipase (MAGL), a key 2-AG–degrading enzyme (19), are lean even though presenting manyfold increased brain 2-AG levels. Similarly, synaptic afferentation of orexin neurons is unchanged in the lateral hypothalamus of $MAGL^{-/-}$ mice. These data clearly establish a hierarchical relationship between leptin and endocannabinoids, with leptin signaling (or the lack thereof) driving synapse remodeling. However, orexin expression was dramatically reduced by a single dose of a CB_1R antagonist in *ob/ob* mice. This result suggests that CB_1Rs on orexin-containing neurons are poised to regulate orexin expression via a hitherto unexplained mechanism.

This research, like any other comprehensive report, inspires a number of radical hypotheses, and certainly calls for further analysis. Neuropeptides coexist with fast neurotransmitters in this arcuate nucleus-lateral hypothalamus circuit (10). A key take-home message of Cristino et al. (5) and earlier studies (3, 4) is that fast neurotransmission, once reorganized, can reset the excitability of neuropeptidergic neurons. Although the relationship of neuropeptide and fast neurotransmitter action remains elusive, an appealing hypothesis is that a floating circuit code driven by the extreme plasticity of GABA/glutamate synapses (3, 4) encodes a form of "metabolic memory" to set the threshold for neuropeptide release.

High-fat diet-induced leptin resistance was found restricted to arcuate neurons, even though the leptin receptor is expressed in many neurons of the lateral hypothalamus (20). This differential response may be a result of the molecular diversity of signal transduction cascades, differences in excitability in vivo, or feedback coupling between leptin and endocannabinoid signaling systems.

The balance between excitation and inhibition on orexin neurons hinges on enhanced DAGLα synthesis in *ob/ob* mice. The fundamental importance of DAGLα in this monosynaptic circuitry could be tested in DAGLα null mice, where synaptic reorganization but not muted inhibition onto orexin-containing neurons would be the anticipated phenotype. More importantly, mechanistic insights in this report suggest that DAGLα inhibitors, rather than CB_1R antagonists, could be used to reinstate inhibition of orexin-containing neurons. If so, this process could facilitate a sea-change in existing "CB_1R-centric" views of weight control, and identify DAGLα as an equally potent molecular target. The ultimate benefit, learning from the failure of rimonabant, is that depressive/anxiety side-effects might be reduced and drug dosing made safer.

Cristino et al. identify an excitatory-to-inhibitory switch of synapses impacting orexin-containing neurons in genetically leptin deficient *ob/ob* mice.

A remarkable finding is that orexin expression in various target areas of the brain was vastly enhanced, fueling the hypothesis that increased orexin release from tegmental and hypothalamic projections will exacerbate obesity. Nevertheless, orexins are primarily implicated in the regulation of arousal and sleep (21), and perhaps in reward aspects via the mesolimbic system (22). Thus, obesity-driven synaptic reorganization in the hypothalamus could also influence narcolepsy (6) and cyclic or bipolar vegetative functions, providing stepping stones to understand the molecular pathology of obesity-linked psychiatric disorders.

Exquisitely designed and executed experiments in rodents, such as the study by Cristino et al. (1), are indispensable to unravel key pathomechanisms of human diseases. Nevertheless, their human relevance, ingrained in potential evolutionary differences in the complexity of underlying neuronal circuitries, must be addressed. Does synapse remodeling on orexin neurons occur in obese humans? The discovery of equivalent patterns and mechanisms will ultimately define the clinical relevance of this study. Alas, the proof of the pudding, or rather a good burger, will be eating it.

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