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Gonadotropin-Releasing Hormone Fibers Contact POMC Neurons in the Hypothalamic Arcuate Nucleus

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Abstract

The metabolic state has long been shown to affect reproduction. Peripheral signals and hormones from the reproductive organs are also known to regulate energy metabolism and feeding and energy expenditure. Much attention has been paid to determine the signaling flow from key hypothalamic neuronal populations, including those producing the anorexigenic proopiomelanocortin (POMC) derivate, α-melanocyte stimulating hormone (α-MSH), to the medial preoptic area gonadotropin-releasing hormone (GnRH) neurons, cells that are the drivers of ovulation and reproduction in general. In this study, the authors explored whether a reverse signaling modality may also exist. Specifically, the authors analyzed GnRH efferents in the arcuate nucleus with particular emphasis on their anatomical proximity to arcuate nucleus melanocortin perikarya. Using correlated light and electron microscopy, the authors observed direct apposition between GnRH-containing axon terminals and POMC cell bodies. These data provide the first experimental evidence to suggest that GnRH may have a direct influence on feeding, energy expenditure, and glucose homeostasis, independent of the activity of the gonadal axis.

Keywords

reproduction, metabolism, hypothalamus, neuroanatomy, electron microscopy

Introduction

A characteristic phenotype of negative energy balance is the hypothalamic hypogonadism.[1,2] This condition is reversible on recovery of energy stores. This evolutionary adaptation allows an individual or species to survive when food is scarce.[3] It is particularly important in female reproduction; a critical fat mass, >10%, is required for ovulation to occur in women.[4] Hypogonadotrophic hypogonadism is also a common phenotype in obesity caused by genetic deficiencies in leptin signaling,[5,6] a hormone that arises from the white adipose tissue and promotes satiety.[7] The fact that reproductive dysfunction in *ob/ob* mice, which have no circulating leptin, can be reversed by leptin treatment indicates that developmental abnormalities in brain structures involved in reproduction caused by leptin deficiency are minimal, if not at all.[8] Similarly, leptin treatment restores gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) secretion and pubertal development in leptin-deficient patients, confirming its critical role in the hypothalamic control of reproduction in humans.[9] Leptin pulsatility is positively and strongly correlated with LH and estrogen levels in normal cycling women,[10] whereas the mean leptin levels and diurnal leptin rhythm are impaired in women with hypothalamic amenorrhea.[11,12] Leptin also restores reproductive function in food-deprived animals and humans, even though their energy stores are not necessarily improved. Leptin accelerates the onset of sexual maturation in ad libitum fed postnatal mice.[13,14] More recently, recombinant leptin was reported to increase the mean LH levels and LH pulse frequency as well as estrogen levels. It improved reproduction in women with hypothalamic amenorrhea.[15] Leptin replacement also restores the pituitary–gonadal axis in patients with lipodystrophia.[16] Leptin directly acts within the hypothalamus to stimulate GnRH secretion in vivo in rats.[17] These findings

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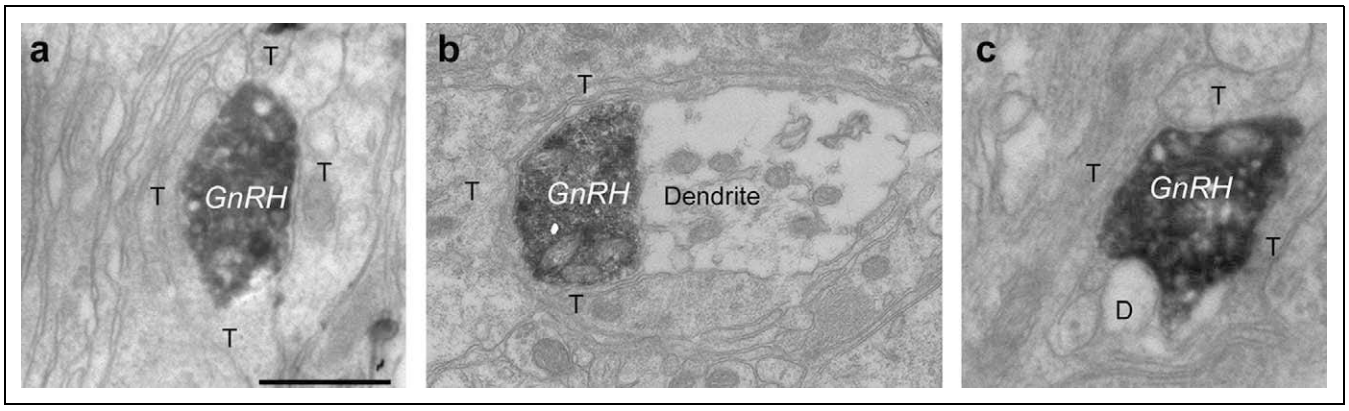

Figure 1. Electron microscopy of GnRH fibers in the arcuate nucleus. A, The vast majority of GnRH-immunoreactive axons travel through the arcuate nucleus enroute to the median eminence surrounded by tanycytic processes (T). Occasionally, however, the ensheathment of axons by tanycytes is interrupted, and GnRH axons with synaptic vesicles can be observed in direct apposition to neuronal profiles, including dendrites (B) or dendritic spines (C). Bar scale on A represents 1 μm for A-C. GnRH indicates gonadotropin-releasing hormone.

suggest that leptin functions as an energy indicator required for normal hypothalamic control of reproduction. When leptin signaling is diminished, the brain switches off the GnRH and LH cycles, resulting in hypogonadotropic hypogonadism and infertility. The primary promoter of GnRH activity triggered by leptin are the arcuate nucleus proopiomelanocortin (POMC)-producing cells located in the arcuate nucleus.[6,18] These neurons are directly connected to GnRH cells.[19]

Gonadotropin-releasing hormone neurons are unique cells in the hypothalamus, as they arise from the olfactory placode and migrate to the preoptic area (POA)—diagonal band of Broca region of the forebrain.[20] They send projections to the external layer of the median eminence located in the mediobasal hypothalamus, where they release GnRH into the portal circulation to alter LH release in the anterior pituitary.[20] There is very little information available regarding the possibility of axon collateral of GnRH neurons affecting any other system but the anterior pituitary. The current study was undertaken to determine whether GnRH axon terminals may establish direct contacts with arcuate nucleus POMC neurons, thereby providing a direct connection between these principal cells in reproduction, with the neurons responsible for feeding, energy expenditure, and glucose homeostasis regulation.[18,21]

Materials and Methods

All procedures described in this article were approved by the Yale Institutional Animal Care and Use Committee. We analyzed female mice (n = 5) that express green fluorescent protein (GFP) in POMC neurons.[22] Correlated light and electron microscopy of GnRH-immunolabeled axons and GFP-labeled (POMC-producing) perikarya was carried out according to our standard, published protocol.[23,24] In this, GnRH was visualized using sheep anti-GnRH antiserum, whereas GFP was labeled using a mouse anti-GFP monoclonal antibody. Labeling of GnRH was accomplished using nickel ammonium sulphate-conjugated diaminobenzidine (DAB), whereas GFP-labeling (POMC cells) was done using DAB. Light microscopical detection of GnRH boutons on POMC cells were processed for electron microscopy. Serial sectioning of this material allows conclusions to be made regarding direct apposition. Control experiments included single staining studies (for either GnRH or GFP), which resulted in very different labeling pattern in the arcuate nucleus.

Results

In accordance with previous observations, GnRH-immunolabeled profiles were visible throughout the arcuate nucleus. Under the electron microscope, most of the GnRH fibers were detected in association with glial profiles most likely tanycytes en route to their final destination, the portal vessels of the external layer of the median eminence (Figure 1).

In the double-labeled material, there were several instances in which POMC perikarya was seen to be in direct apposition to GnRH-immunolabeled boutons (Figure 2). Of the 100 POMC neurons detected in 5 animals, 25 POMC immunoreactive cell bodies and/or proximal dendrites were found to be contacted by GnRH-labeled axons.

When correlated electron microscopy was carried out, all of these connections appeared to be direct physical appositions between GnRH-producing axon terminals and POMC-labeled perikarya (Figure 2). However, none of the connections that were analyzed by electron microscopy were classical synaptic membrane specializations.

Discussion

Like leptin, the gonadal steroid hormone, estrogen, reduces food intake and body adipocytes and increases energy expenditure in animals and humans of both genders through a hypothalamic mechanism.[18,25-27] It exerts a profound impact on reproduction both peripherally and centrally. The central effect of estrogen in the regulation of reproduction is directly related to reproductive hormone cycles. In fact, the actions of estrogen on the hypothalamic GnRH neuronal network are required to

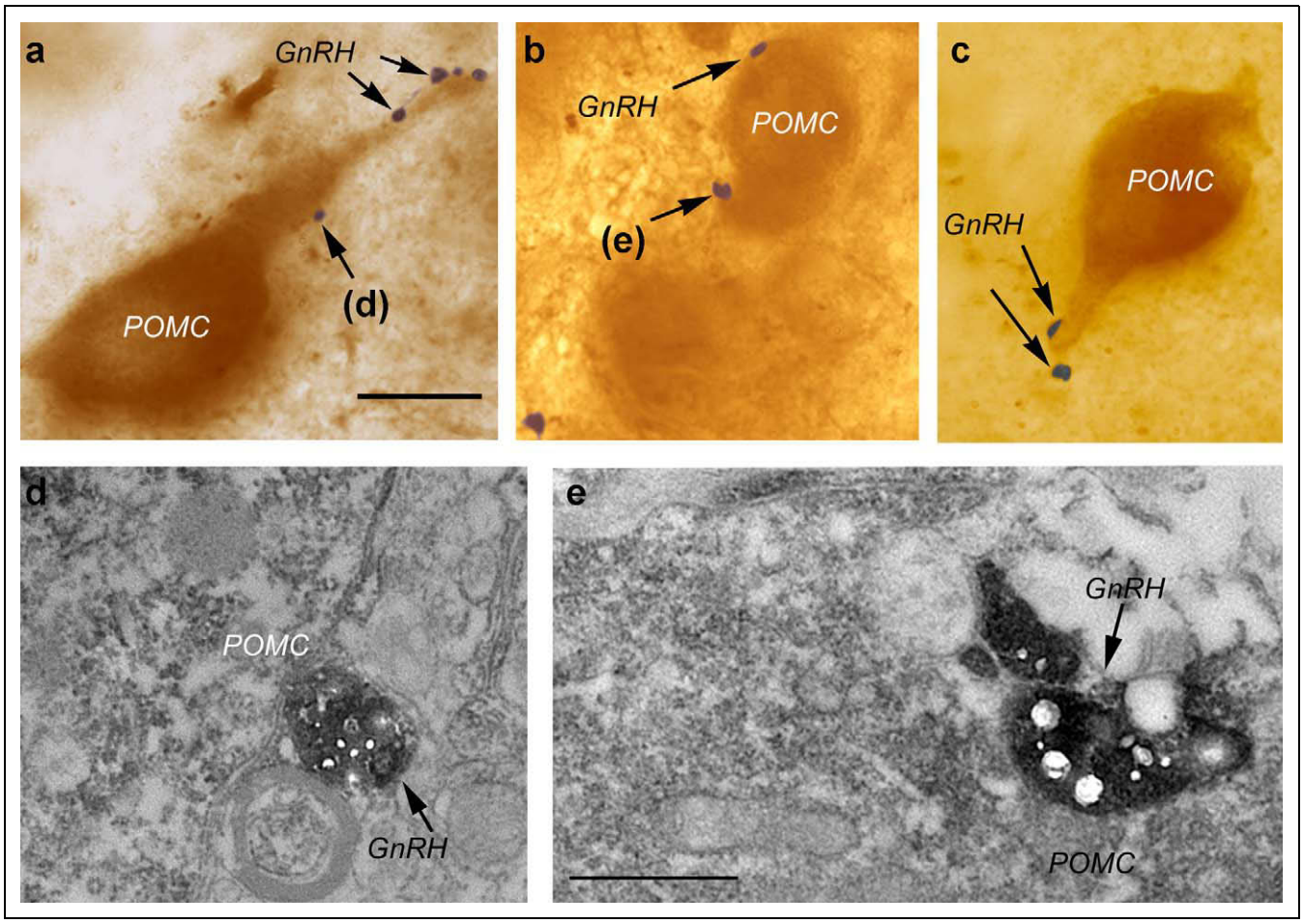

Figure 2. GnRH contacts on POMC cells (A-C). Using double immunolabeling, we observed a preferential interaction between putative GnRH boutons (blue boutons indicated by black arrows) and arcuate nucleus POMC-producing neurons (light brown profiles). (d) on A and (e) on B indicate boutons that are processed for electron microscopy and shown on panels D and E. Bar scale on A indicates 10 μm for panels A-C. D and E, Electron microscopic analysis of putative contacts indicated on panels A and C revealed that GnRH-immunolabeled axons (black arrows) were in direct apposition to perikaryal membranes of POMC neurons, without interposition by glial processes. Bar scale on E represents 1 μm for D and E. GnRH indicates gonadotropin-releasing hormone; POMC, proopiomelanocortin.

trigger the episodic release of GnRH that leads to a pulsatile pattern of LH secretion.[28] The ability of leptin to restore fertility is also achieved by restoring GnRH and LH surges, which, in turn, reverse hypogonadotropic hypogonadism. In adult females, circulating estrogen is mainly produced in the ovary. Locally, estrogen can be converted from testosterone and androstenedione by the enzyme cytochrome P450 aromatase (P450aro). The levels of P450aro in male brains are slightly higher than in females;[29-31] however, aromatase-deficient mice accumulate excess adipose tissue in both genders[32] and impairs male fertility.[33,34] In humans, aromatase deficiency has been associated with hypogonadotropic hypogonadism and infertility in both genders,[35,36] suggesting that local estrogen is important for homeostasis and reproduction. Consistent with this, P450aro is highly and selectively expressed in the regions associated with metabolism, that is, ventromedial nucleus of the hypothalamus (VMH) and reproduction, that is, POA.[37]

The aforementioned information reveals that both gonadal input and the metabolic state via hormonal signals, such as leptin, have profound influence on GnRH neuronal activity and, via this mechanism, on the gonadal axis. However, to date, no attempts have been made to determine whether a signaling modality from GnRH neurons to melanocortin cells may also exist, thereby providing a primary reproductive efferent input to cells that govern feeding and energy expenditure. Our results reveal that such information flow may, in fact, exist. We established with correlated light and electron microscopic analysis that GnRH efferents make direct contacts with POMC perikarya in the hypothalamic arcuate nucleus. Although we could not detect synaptic membrane specializations between these neuronal elements, the fact that these synaptic boutons are in direct physical contact with each other provides the morphological substrate for these axons to affect the electric properties of POMC cells. Further studies are needed to determine whether these connections have stimulatory or inhibitory effects on POMC neurons firing, and whether this novel signaling modality have an impact on functional aspects of the melanocortin system.

Declaration of Conflicting Interests

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article.

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