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Melanin concentrating hormone and estrogen receptor- α are coextensive but not coexpressed in cells of male rat hypothalamus

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Abstract

In male rats, estradiol (E_2) exerts marked anorectic effects. One mechanism proposed for this effect is an E_2 -mediated down-regulation of the orexigenic neuropeptide melanin concentrating hormone (MCH). Previous anatomical work has shown that both MCH and estrogen receptor α (ER α) are found in quantity in the lateral hypothalamic area (LHA), a structure long associated with appetite and ingestive behavior. It has been hypothesized that the most direct manner by which E_2 could affect MCH expression and feeding would be via classical nuclear ER α located in MCH neurons. To evaluate this notion, we performed double-label immunohistochemistry for MCH and ER α in male rat hypothalamus. We report here that MCH neurons do not contain ER α , suggesting that the primary locus for estrogenic control of feeding is not the MCH neurons themselves. Rather, we show substantial overlap in the anatomical distribution of both cell types, raising the possibility that E_2 influences MCH signaling indirectly via adjacent ER α -containing cells.

Keywords

Anorexia; estradiol; ingestive behavior; neuropeptides; gonadal steroids; hypothalamus

Since its discovery in neurons of the mammalian hypothalamus [3,26], melanin concentrating hormone (MCH) has been studied as a key regulator of energy balance [16]. Central administration of MCH promotes feeding [5,17,18], while genetic ablation of the *Mch* gene produces a lean phenotype often characterized by hypophagia [1,21]. It has also been demonstrated that the *Mch* gene is up-regulated as a compensatory response to energy restriction [17]. According to one model for the hypothalamic control of food-intake and energy balance, MCH-containing neurons near the lateral hypothalamic area (LHA) are “second order” neurons, once removed from the site of adiposity peptide (i.e. leptin) signaling in the arcuate nucleus [8,20,27]. Anatomical data suggest that a condition of negative energy balance is relayed to MCH cells via orexigenic neuropeptide Y (NPY)- and agouti-related protein (AgRP)-containing neurons, while the opposite condition is signaled by neurons containing the anorectic peptides proopiomelanocortin (POMC) and α -melanocyte stimulating hormone (α -MSH) [4,6,7]. MCH-containing neurons presumably integrate this information from the

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arcuate, organizing feeding behavior accordingly via their projections to brain structures like the nucleus accumbens [9].

While increased MCH activity appears to bias animals towards attaining a state of positive energy balance, estradiol (E₂) has the opposite effect, producing weight loss and aphagia when given at supraphysiologic doses [2,14]. Additionally, genetic ablation of estrogen receptor α (ER α) produces an obese phenotype [10]. The presence of ER α in LHA, near the main population of MCH-containing neurons, and in arcuate nucleus has prompted investigations of whether this peptide mediates the aphagic effects of E₂ [13,15], presumably by impairing MCH signaling. In male rats, *Mch* expression decreases markedly with E₂ administration [15], and it has been suggested that this change may be responsible for E₂'s anorexic effects. The most straightforward manner in which E₂ could affect *Mch* expression would be via action at nuclear estrogen receptors located in MCH neurons. Alternatively, the presence of estrogen receptors in neighboring cells of the LHA or arcuate would indicate an indirect, afferent-driven mechanism of *Mch* regulation. To determine the anatomical relationship between ER α and MCH in the hypothalamus, we performed double-label immunohistochemistry for these substances and mapped their distribution in male rats.

In this experiment, 40 μ m paraformaldehyde-fixed, frozen sections from hypothalami of four adult (~350 g) male Long-Evans rats (Harlan, Indianapolis, IN, kept and used in accordance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals*), were first labeled for ER α (1:1,000, Santa Cruz Biotechnology, Santa Cruz, CA) with goat anti-rabbit secondary antibody (1:600, Vector, Burlingame, CA) and visualized using nickel-intensified 3'3'-diaminobenzidine (DAB) and an avidin-biotin peroxidase kit (Elite Kit, Vector), according to procedures described previously [19]. Because there is little evidence for the expression of ER β near the MCH neuronal field [11], labeling for that receptor subtype was not performed. After ER α labeling was complete and tissue was reblocked with normal goat serum, a second round of labeling was conducted for MCH (1:2,500, Phoenix Pharmaceuticals, Belmont, CA, secondary antibody as above) using only DAB as the chromogen. Serial double immunolabeling revealed a pattern of opaque, black, nuclear ER α label and translucent brown labeling in cytosol for MCH. In no case were antigens found outside their expected cellular compartments (e.g. black label in cytosol or brown label in nucleus). Control sections from each animal were incubated in absence of primary antibodies for MCH, ER α , or both, and in all cases labeling for that antigen was absent. Major landmarks and labeled cells from each of six consistent rostrocaudal levels of hypothalamus were drawn using a microscope and camera lucida. These drawings were digitally scanned and transposed using Adobe Illustrator software to corresponding levels of a reference atlas [23].

Anatomical distribution of each antigen was consistent with previously published research reports using single-label immunohistochemistry or *in situ* hybridization [3,22,24] (Figures 1A, 2). Both MCH- and ER α -labeled cells were sparse at the level of the paraventricular nucleus (PVN). Most MCH-labeled neurons there appeared dorsally in zona incerta (ZI), above the fornix and stretching medially to the third ventricle. ER α -labeled cells appeared in the ventrolateral aspect of the ventromedial hypothalamic nucleus (VMH) and arcuate nucleus, and ascended along the ventricle through the periventricular area, eventually mingling with MCH-labeled neurons in PVN. More caudally, as each cell type became more numerous, this general pattern continued, but ER α -labeled cells also occurred alongside MCH neurons in the ZI and LHA. Interestingly, in no case were MCH neurons seen to express ER α (Figure 1B). It is also notable that MCH neurons avoided the dorsomedial hypothalamic nucleus (DMH) and VMH, often appearing between the two structures as the population stretched medially to near the third ventricle (Figure 2). Together, these observations present a heterogeneous picture of MCH and ER α distribution in hypothalamus where there are areas of overlap (LHA, PVN, ZI) as well as partial exclusion (ER α but not MCH in VMH).

The absence of nuclear ER α in MCH neurons may help to elucidate the mechanism of estradiol-induced anorexia. Previously it was assumed that E₂ must act directly at estrogen receptors in MCH neurons to reduce expression of that peptide, thereby inducing aphagia [15]. Indeed, this model is parsimonious and has intuitive appeal. Our data suggest a more complex model, where E₂ may still act locally in the hypothalamus, particularly in those structures where ER α - and MCH-containing cells are found to mingle. Ultimately, regulation of MCH expression and appetite by ER α and its ligand E₂ appears to be indirect and not within MCH neurons themselves. Given their close proximity, it seems possible that local afferents from ER α -containing cells synapse on MCH neurons to affect their electrical properties and transcriptional activity. Additional anatomical work will be required to confirm this synaptic arrangement and to identify transmitters used to signal between ER α -expressing cells and neighboring MCH neurons. One might anticipate, however, that ER α -containing cells would convey a net-inhibitory signal on both domains of MCH neuronal function.

While these data suggest an indirect mechanism for the previously described regulation of appetite and *Mch* expression by E₂, we have not overlooked the possibility that more direct action of E₂ on MCH neurons may occur via novel, non-genomic means. In recent years, evidence has accumulated to support the existence of extra-nuclear, membrane-bound forms of estrogen receptors [12]. Such receptors can exert potent effects, not only on neuronal excitability, but also transcriptional activity [25]. Though rapid effects of E₂ have not been described in MCH neurons, and we report no extranuclear labeling by the ER α antibody we used, this mechanism cannot be ruled out.

In summary, our data suggest that the most likely substrates for the regulation of ingestive behavior by MCH and ER α are structures where those substances are found in close association (e.g. LHA). Additionally, estrogenic regulation of MCH-induced ingestive behavior appears to occur at least one synapse removed from MCH neurons themselves, as these neurons do not express ER α .

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References

1. Alon T, Friedman JM. Late-onset leanness in mice with targeted ablation of melanin concentrating hormone neurons. *J Neurosci* 2006;26:389–97. [PubMed: 16407534]
2. Bernstein IL, Courtney L, Braget DJ. Estrogens and the Leydig LTH(m) tumor syndrome: anorexia and diet aversions attenuated by area postrema lesions. *Physiol Behav* 1986;38:159–63. [PubMed: 3467386]
3. Bittencourt JC, Presse F, Arias C, Peto C, Vaughan J, Nahon JL, Vale W, Sawchenko PE. The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. *J Comp Neurol* 1992;319:218–45. [PubMed: 1522246]
4. Broberger C, de Lecea L, Sutcliffe JG, Hokfelt T. Hypocretin/orexin- and melanin-concentrating hormone-expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to the neuropeptide Y and agouti gene-related protein systems. *J Comp Neurol* 1998;402:460–474. [PubMed: 9862321]
5. Della-Zuana O, Presse F, Ortola C, Duhault J, Nahon JL, Levens N. Acute and chronic administration of melanin-concentrating hormone enhances food intake and body weight in Wistar and Sprague-Dawley rats. *Int J Obes Relat Metab Disord* 2002;26:1289–95. [PubMed: 12355323]
6. Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, Elmquist JK. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron* 1999;23:775–86. [PubMed: 10482243]

7. Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J, Tatro JB, Hoffman GE, Ollmann MM, Barsh GS, Sakurai T, Yanagisawa M, Elmquist JK. Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. *J Comp Neurol* 1998;402:442–459. [PubMed: 9862320]
8. Flier JS. Obesity wars: molecular progress confronts an expanding epidemic. *Cell* 2004;116:337–50. [PubMed: 14744442]
9. Georgescu D, Sears RM, Hommel JD, Barrot M, Bolanos CA, Marsh DJ, Bednarek MA, Bibb JA, Maratos-Flier E, Nestler EJ, DiLeone RJ. The hypothalamic neuropeptide melanin-concentrating hormone acts in the nucleus accumbens to modulate feeding behavior and forced-swim performance. *J Neurosci* 2005;25:2933–40. [PubMed: 15772353]
10. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. *Proc Natl Acad Sci U S A* 2000;97:12729–34. [PubMed: 11070086]
11. Li X, Schwartz PE, Rissman EF. Distribution of estrogen receptor-beta-like immunoreactivity in rat forebrain. *Neuroendocrinology* 1997;66:63–7. [PubMed: 9263202]
12. Losel RM, Falkenstein E, Feuring M, Schultz A, Tillmann HC, Rossol-Haseroth K, Wehling M. Nongenomic steroid action: controversies, questions, and answers. *Physiol Rev* 2003;83:965–1016. [PubMed: 12843413]
13. Messina MM, Boersma G, Overton JM, Eckel LA. Estradiol decreases the orexigenic effect of melanin-concentrating hormone in ovariectomized rats. *Physiol Behav* 2006;88:523–8. [PubMed: 16793070]
14. Mordes JP, Longcope C, Flatt JP, MacLean DB, Rossini AA. The rat LTW(m) Leydig cell tumor: cancer anorexia due to estrogen. *Endocrinology* 1984;115:167–73. [PubMed: 6587964]
15. Mystkowski P, Seeley RJ, Hahn TM, Baskin DG, Havel PJ, Matsumoto AM, Wilkinson CW, Peacock-Kinzig K, Blake KA, Schwartz MW. Hypothalamic melanin-concentrating hormone and estrogen-induced weight loss. *J Neurosci* 2000;20:8637–42. [PubMed: 11069973]
16. Pissios P, Bradley RL, Maratos-Flier E. Expanding the scales: The multiple roles of MCH in regulating energy balance and other biological functions. *Endocr Rev* 2006;27:606–20. [PubMed: 16788162]
17. Qu D, Ludwig DS, Gammeltoft S, Piper M, Pellemounter MA, Cullen MJ, Mathes WF, Przypek R, Kanarek R, Maratos-Flier E. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature* 1996;380:243–7. [PubMed: 8637571]
18. Rossi M, Choi SJ, O'Shea D, Miyoshi T, Ghati MA, Bloom SR. Melanin-concentrating hormone acutely stimulates feeding, but chronic administration has no effect on body weight. *Endocrinology* 1997;138:351–5. [PubMed: 8977423]
19. Sato S, Braham CS, Putnam SK, Hull EM. Neuronal nitric oxide synthase and gonadal steroid interaction in the MPOA of male rats: co-localization and testosterone-induced restoration of copulation and nNOS-immunoreactivity. *Brain Res* 2005;1043:205–13. [PubMed: 15862534]
20. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000;404:661–71. [PubMed: 10766253]
21. Shimada M, Tritos NA, Lowell BB, Flier JS, Maratos-Flier E. Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature* 1998;396:670–4. [PubMed: 9872314]
22. Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. *J Comp Neurol* 1990;294:76–95. [PubMed: 2324335]
23. Swanson, LW. A Laboratory Guide with Printed and Electronic Templates for Data, Models and Schematics. Vol. 3. Elsevier; 2004. *Brain Maps III: Structure of the Rat Brain*.
24. Swanson LW, Sanchez-Watts G, Watts AG. Comparison of melanin-concentrating hormone and hypocretin/orexin mRNA expression patterns in a new parceling scheme of the lateral hypothalamic zone. *Neurosci Lett* 2005;387:80–4. [PubMed: 16084021]
25. Vasudevan N, Kow LM, Pfaff D. Integration of steroid hormone initiated membrane action to genomic function in the brain. *Steroids* 2005;70:388–96. [PubMed: 15862822]
26. Vaughan JM, Fischer WH, Hoeger C, Rivier J, Vale W. Characterization of melanin-concentrating hormone from rat hypothalamus. *Endocrinology* 1989;125:1660–5. [PubMed: 2759038]

27. Zigman JM, Elmquist JK. Minireview: From anorexia to obesity--the yin and yang of body weight control. *Endocrinology* 2003;144:3749–56. [PubMed: 12933644]

Figure 1.

A) Micrograph of MCH and ER α immunopositive cells in hypothalamus 3.25 mm caudal to bregma. ER α -labeled nuclei are most apparent in arcuate nucleus and VMH. MCH-labeled neurons are most apparent in LHA. Dashed box indicates area of magnification. Scale bar = 100 μ m. B) Higher magnification detail of ER α -positive nuclei (arrows) and MCH-positive neurons (brown cytosolic label). Scale bar = 100 μ m. Arc, arcuate nucleus; DMH, dorsomedial hypothalamic nucleus; fx, fornix; LHA, lateral hypothalamic area; 3v, third ventricle.

Figure 2.

Representative map of MCH- (open circles) and ER α - (closed circles) labeled cells in male rat hypothalamus. Numbers in lower left are mm caudal to bregma. AHN, anterior hypothalamic nucleus; cpd, cerebral peduncle; PH, posterior hypothalamic nucleus; PVN, paraventricular hypothalamic nucleus; ZI, zona incerta.