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Central Serotonin and Melanocortin Pathways Regulating Energy Homeostasis

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ABSTRACT: It is now established that the hypothalamus is essential in coordinating endocrine, autonomic, and behavioral responses to changes in energy availability. However, the interaction of key peptides, neuropeptides, and neurotransmitters systems within the hypothalamus has yet to be delineated. Recently, we investigated the mechanisms through which central serotonergic (5-hydroxytryptamine, 5-HT) systems recruit leptin-responsive hypothalamic pathways, such as the melanocortin systems, to affect energy balance. Through a combination of functional neuroanatomy, feeding, and electrophysiology studies in rodents, we found that 5-HT drugs require functional melanocortin pathways to exert their effects on food intake. Specifically, we observed that anorectic 5-HT drugs activate pro-opiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus (Arc). We provide evidence that the serotonin 2C receptor (5-HT_{2C}R) is expressed on POMC neurons and contributes to this effect. Finally, we found that 5-HT drug-induced hypophagia is attenuated by pharmacological or genetic blockade of downstream melanocortin

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3 and 4 receptors. We review candidate brain regions expressing melanocortin 3 and 4 receptors that play a role in energy balance. A model is presented in which activation of the melanocortin system is downstream of 5-HT and is necessary to produce the complete anorectic effect of 5-HT drugs. The data reviewed in this paper incorporate the central 5-HT system to the growing list of metabolic signals that converge on melanocortin neurons in the hypothalamus.

KEYWORDS: serotonin; melanocortin; pro-opiomelanocortin (POMC); serotonin 2C receptor (5-HT_{2C}R); melanocortin 4 receptor (MC4-R); food intake; body weight; hypothalamus; and arcuate nucleus

Obesity and type II diabetes are rising at alarming rates in the United States.[1] Elucidating the basic neurobiology of energy homeostasis is paramount in the prevention and treatment of these conditions.[2] Over the past decade, remarkable progress has been made in the understanding of how the central nervous system (CNS), especially the hypothalamus, controls food intake and body weight homeostasis. In the short review that follows, we outline some recent advances in the hypothalamic mechanisms involved in regulating energy balance.

The central serotonin (5-hydroxytryptamine, 5-HT) system has been long been associated with food intake and body weight regulation. Pharmacological agents that increase 5-HT activity in the CNS inhibit food intake and promote weight loss.[3–5] However, the 5-HT neural circuits underlying energy homeostasis have been difficult to discern because of the widespread nature of 5-HT neuronal projections, the identification of at least 14 distinct 5-HT receptors, and the relative paucity of receptor-selective drugs.[6] The inability to pharmacologically target specific 5-HT pathways and receptors has contributed to the incidence of unwanted side-effects with anorectic 5-HT indirect agonists. A notable example is d-fenfluramine (d-Fen), a drug that blocks the reuptake of 5-HT and stimulates its release.[7,8] In the mid-1990s, d-Fen was prescribed to millions of people in the United States for weight loss, frequently in combination with phentermine (fen/phen), but was withdrawn from clinical use in 1997 by the Food and Drug Administration due to reports of adverse cardiopulmonary events.[9]

Recently, we have undertaken a series of experiments in an effort to determine specific CNS pathways through which d-Fen selectively reduces food intake and body weight. d-Fen dose-dependently induces Fos-like immunoreactivity (FOS-IR), a commonly used marker of neuronal activation, in many brain regions associated with energy regulation.[10] We observed significant FOS-IR induction in the lateral, but not medial, arcuate nucleus of the hypothalamus (Arc) of the rat.[10] This pattern of FOS-IR induction is consistent with the distribution of neurons containing the anorectic neuropeptides pro-opiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART).[11] Additional evidence that 5-HT acts on POMC neurons is found when examining 5-HT neuronal projections and receptor expression patterns. Specifically, 5-HT-immunoreactive nerve terminals contact POMC neurons in the Arc.[12] 5-HT drugs also cause the release of the protein product of POMC, α-melanocyte stimulating hormone (α-MSH) from superfused hypothalamic slices.[13] 5-HT receptor distribution studies indicate that at least four 5-HT receptors are expressed in the Arc.[14–16] These data suggest that the central 5-HT system is positioned to act on POMC neurons.

Activation of POMC neurons causes the release of the endogenous melanocortin receptor agonist α-MSH. Centrally administered α-MSH reduces food intake, while treatment with the endogenous melanocortin receptor antagonist agouti-related protein (AgRP) increases feeding.[17,18] α-MSH and AgRP are expressed in distinct Arc neuronal populations, both of which project to regions implicated in energy homeostasis and autonomic outflow that contain melanocortin 4 receptor (MC4-R) expressing neurons.[11,19–22] Pharmacological blockade or genetic inactivation of MC4-Rs in laboratory animals and human subjects produces hyperphagia, reduced energy expenditure, obesity, and insulin resistance.[23–29] The recent surge of data on the CNS melanocortin pathways indicates that this system is a critical regulator of energy homeostasis.

We propose that 5-HT drugs, such as d-Fen, reduce food intake and body weight by engaging these melanocortin pathways. Support for this hypothesis was found through both *in vivo* and electrophysiological techniques. Specifically, rats treated with anorectic doses of d-Fen exhibited significant FOS-IR induction in POMC neurons at all rostrocaudal levels of the Arc.[10] We also performed electrophysiological recordings in transgenic mice expressing green fluorescent protein under the control of POMC promoter (GFP-POMC).[10,30] These experiments indicate that d-Fen and 5-HT induce highly consistent depolarization of Arc POMC neurons.[10] Taken together, these data provide strong support for a mechanism of d-Fen hypophagia via 5-HT-induced activation of Arc POMC neurons.

Of the 5-HT receptors identified in the Arc, the 5-HT_{2C}R is the most intriguing candidate contributing to the anorectic actions of d-Fen. 5-HT_{2C}Rs have been implicated in the modulation of food intake, body weight, and autonomic function through both transgenic and pharmacological studies.[3,5] 5-HT_{2C}R deficient (–/–) mice are hyperphagic, obese, and display blunted responses to anorectic properties of d-Fen.[31,32] We determined that 5-HT_{2C}R mRNA is co-expressed at a rate up to 80% with α-MSH-containing neurons in the Arc.[10] If d-Fen and 5-HT activate POMC neurons via action at 5-HT_{2C}Rs, then treatment with a 5-HT_{2C}R agonist should mimic the effects of d-Fen and 5-HT in this population of neurons. We found that the 5-$HT_{2C/1B}$R agonist 1-(3-chlorophenyl)piperazine (mCPP) induces dose-dependent FOS-IR in Arc POMC neurons in a similar manner produced by d-Fen.[10] mCPP and the 5-$HT_{2C/2A}$R agonist MK212 also dose-dependently depolarize POMC neurons in GFP-POMC transgenic mice.[10] These results strongly support the hypothesis that 5-HT_{2C}Rs expressed on POMC neurons are involved in hypophagia induced by 5-HT drugs like d-Fen.

If the melanocortin pathway is a critical mediator of 5-HT drug action on energy homeostasis, then it could be hypothesized that blockade of a downstream melanocortin receptor will attenuate these effects. Supporting this hypothesis, experiments with mice ectopically overexpressing the endogenous melanocortin receptor antagonist agouti (Ay mice) show blunted responses to threshold anorectic doses of d-Fen.[10] Similarly, rats pretreated with the MC3-R/MC4-R antagonist SHU9119 also display attenuated responses to d-Fen-induced hypophagia.[10] These results are remarkable in light of the efficacy of d-Fen in other rodent models of obesity, such as the ob/ob mouse.[33] These data demonstrate that the anorectic properties of d-Fen require functional melanocortin pathways. On the other hand, 5-HT_{2C}R-deficient (–/–) mice treated with the MC3-R/MC4-R agonist MTII exhibit reductions in food intake comparable to their wild-type littermates. This latter observation suggests that 5-HT_{2C}R

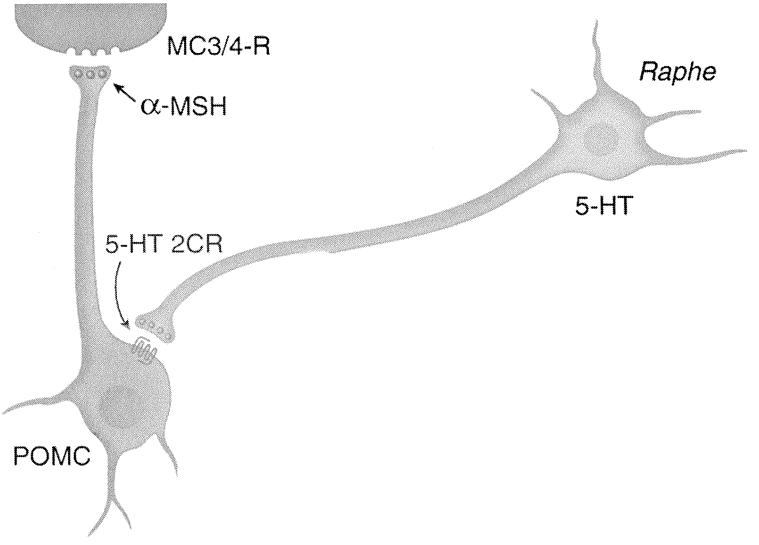

FIGURE 1. Schematic of proposed hypophagic action of 5-HT drugs involving the recruitment of central melanocortin pathways.

action is upstream of MC3-R/MC4-Rs.[10] Taken together, these data lead us to offer a model of the mechanisms of d-Fen-induced hypophagia (FIG. 1). This model predicts that d-Fen increases the availability of 5-HT in the Arc, which acts on POMC neurons through 5-HT_{2C}Rs. This activation of POMC neurons causes the release of α-MSH, which then acts on neurons expressing MC3-Rs and/or MC4-Rs in downstream target CNS regions regulating energy homeostasis.

Candidate CNS regions implicated in feeding behavior that receive dense inputs from Arc POMC and AgRP neurons and contain MC4-Rs and/or MC3-Rs are the paraventricular nucleus of the hypothalamus (PVH) and lateral hypothalamic area (LHA).[34–36] Localized PVH injections of the MC3-R/MC4-R agonist MTII inhibit feeding, while injections of the MC3-R/MC4-R antagonist SHU9119 or MC4-R antagonist HS014 increases food intake.[37–39] Relatively few neurons express MC4-R mRNA in the LHA.[20,21] Most likely, either the MC3-R is a predominant melanocortin receptor regulating feeding behavior in the LHA,[40] or MC4-Rs are expressed on axon terminals (from certain CNS sites projecting to the LHA) and act presynaptically. The dorsal motor nucleus of the vagus (DMV) is another region that contains a high degree of MC4-R mRNA expression that may contribute to the melanocortin regulation of food intake and body weight.[20,21,41]

In summary, the pathophysiological basis for aberrant feeding behavior, body weight regulation, and endocrine function has yet to be determined. Pharmacological agents targeting both the serotonergic and melanocortin pathways are very effective in altering energy balance. In this paper, we propose that the central 5-HT system converges on melanocortin pathways as a final common output mechanism to promote energy homeostasis. We propose that a serotonergic receptor critically in-

volved in this pathway is the 5-HT_{2C}R, and that the MC4-R is a crucial downstream melanocortin receptor affecting energy balance. Future work on this model of energy homeostasis may delineate necessary pathways of serotonergic and melanocortin drug action, and thereby may identify potential selective targets for the prevention and treatment of obesity and type II diabetes.

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