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Central Leucine Sensing in the Control of Energy Homeostasis

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INTRODUCTION

Leucine is an essential branched chain amino acid (BCAA) that drives intracellular signaling cascades critical to protein synthesis and cellular proliferation via the mammalian target of rapamycin complex 1 (mTORC1) - S6K1inase pathway. Both plasma and cerebrospinal leucine levels are rapidly elevated after a high leucine meal [1], and brain access to leucine is mediated by facilitative transport involving both saturable and unsaturable processes [2]. Among amino acid stimuli, l-leucine has been reported to be the most potent activator of p70S6K1 [3]. Phosphorylation of S6 kinase, in turn, has been implicated in the negative feedback control of insulin signaling via insulin receptor substrate 1 (IRS1) [4], supporting a potential role for important interactions between leucine and insulin in determining whole body glucose homeostasis through mTORC1-S6K1. In mice, consitutitive, systemic deletion of S6K1 protects against diet-induced obesity and enhances insulin sensitivity, yet promotes glucose intolerance [4]. In rats, BCAA supplementation of a high fat diet (HFD) reduces weight gain, while increasing insulin resistance, suggesting that in the context of HFD, dietary BCAA contributes to the development of insulin resistance during obesity [5]. However, chronic and specific dietary leucine supplementation during HFD has been shown to markedly reduces hyperglycemia, hypercholesteremia, weight gain and adiposity, while increasing resting energy expenditure and molecular activity of uncoupling protein 3 in brown and white adipose tissue, as well as skeletal muscle, without affecting total daily food intake [6]. These apparently disparate findings have driven an ongoing search for specific sites of leucine and downstream p70S6K1 activation important in the control of multiple effectors of energy balance, including glucose homeostasis, food intake, and adiposity. This manuscript reviews results from recent studies identifying two key brain regions, the mediobasal hypothalamus (MBH, including the arcuate (ARC) and ventromedial (VMN) nuclei) and the dorsal vagal complex of the caudal brainstem (DVC, including the nucleus of the solitary tract, the dorsal motor vagus, and the area postrema), as two critical nodes in the neural network where central leucine sensing contributes to whole body energy homeostasis.

HYPOTHALAMIC LEUCINE AND GLUCOSE HOMEOSTASIS

Important early support for a focus on the central nervous system as a possible site of leucine sensing came from studies of hypothalamic insulin signaling and its role in glucose homeostasis. Results from these studies revealed that central insulin infusions suppressed glucose production during insulin clamp studies, where exogenous glucose is infused to maintain stable glycemia. Central administration of antisense oilgonucleotides directed against insulin receptors selectively decreased insulin receptor expression in the MBH, and significantly attenuated the ability of central insulin to suppress glucose production during a clamp [7]. Together, these data support an important role for MBH insulin receptors in the ability of central insulin to affect endogenous glucose production. Based on the aforementioned negative feedback relationship between p70S6K1 activation and reduced insulin signaling, Ono and colleagues subsequently investigated the degree to which MBH S6K1 activity might also mediate the ability of hypothalamic insulin to determine glucose homeostasis during a clamp. Acute exposure to HFD elevated hypothalamic S6K1 expression, while decreasing central insulin sensitivity. Adenovirally mediated, constitutitive activation of S6K1 within the MBH mimicked the ability of HFD to limit central insulin action in the control of glucose production, while suppression of hypothalamic S6K1 activity by overexpression of a dominant negative form of the kinase partially reversed the effects of HFD [8].

Results of these findings supported the possibility that MBH leucine itself, as a potent stimulus of mTOR and S6K1 phosphorylation, would also contribute to the control of glucose homeostasis. Indeed, MBH leucine infusion alone significantly lowered both plasma and glucose levels, and during a basal clamp, MBH leucine suppressed hepatic glucose production by decreasing both glycogenolysis and gluconeogenesis [9]. Pharmacological or viral blockade of leucine metabolism within the MBH also blocked the ability of MBH leucine to suppress glucose production[9]. Taken together, these data support a specific role for MBH leucine metabolism in the control of glucose homeostasis.

HYPOTHALAMIC LEUCINE AND FOOD INTAKE

Initial studies by Cota et al. [10] demonstrated an important role for central leucine and its downstream targets mTOR and S6K1 in the control of food intake and body weight. These studies were advanced by findings that the mTOR-S6K1 pathway was rapidly and robustly activated selectively within MBH ARC neurons expressing anorexigenic pro-opiomelanocoritn (POMC) and orexigenic neuropeptide y (NPY)/agouti related peptide (AGRP) neurons within the ARC) during refeeding after a fast. In contrast, hypothalamic paraventricular nucleus (PVN) mTOR and S6K1 activity was unchanged, suggesting that dietary nutrients selectively activated the mTOR-S6K1 pathway within ARC neurons of the MBH. In subsequent studies in fasted rats, they found that third intracerebroventricular (3icv) administration of leucine rapidly stimulated S6K1 activation within the MBH, and reduced food intake as early as 4 hours after injection. This reduction persisted for 24 h, resulting in lower weight gain relative to vehicle treated controls. As the MBH is adjacent to the third ventricle site of leucine administration, these data supported the possibility that leucine acted directly on ARC MBH neurons to reduce feeding and body weight gain. In contrast, 3icv administration of l-valine, a non-ketogenic branched chain amino acid used as a control for the chemospecificity of leucine's effects, failed to alter food intake or body weight gain.

The mTOR-S6K1 pathway plays an important role in the ability of 3icv leucine to affect energy intake, as co-adminisrtation of leucine with subthreshold doses the mTOR inhibitor rapamycin, that had no effect on feeding when administered alone, blocked leucine's effects.

Higher doses of rapamycin alone were sufficient to rapidly increase food intake, supporting a role for endogenous leucine sensing in the control of energy balance via mTOR-S6K1 [10]. Taken together, these data have been interpreted to support the suggestion that central leucine's feeding suppressive and metabolic effects are mediated by the MBH. However, these studies did not directly challenge this suggestion in two important ways. First, the 3icv administration protocols employed did not exclusively target the MBH, and second, there was limited investigation of non-hypothalamic sites, leaving unaddressed the possibility that central leucine sensing at extrahypothalamic sites may also be important in the control of energy balance.

Subsequent studies by Blouet and colleagues addressed both of these concerns, identifying specific roles for MBH leucine and mTOR-S6K1 in the control of energy balance, and revealing the importance of hitherto unreported brainstem leucine sensing capabilities. Selective MBH application of l-leucine or equimloar doses of its ketoacid, α-ketoisocaproic acid (KIC), in mice and rats, reduced both short term feeding, 24 h cumulative intake, and 24 h body weight gain, without affecting energy expenditure, locomotor activity, core temperature, or oxygen consumption. In contrast, neither MBH l-valine nor MBH administration of its ketoacid, α-ketoisovaleric acid (KIV), had any effect, consistent with the chemospecificity of MBH l-lecuine sensing in the hypothalamic control of energy balance. Furthermore, MBH leucine did not support the formation of a conditioned taste aversion. These findings support a feeding specific action of MBH leucine in the control of body weight. Chronic, site-specific pharmacological inhibition of leucine oxidative metabolism recapitulated the feeding and body weight reductions seen in response to leucine and KIC. The reductions in feeding observed following MBH leucine were due to a selective reduction in meal size, with no acute change in meal frequency, implicating neuronal pathways important in the control of meal size, especially the brainstem, in the feeding suppressive actions of MBH leucine. Taken together, these data: 1) support a role for endogenous leucine metabolism at the MBH in the control of food intake body weight gain, and 2) implicate neural substrates important in the control of meal size as likely components of the neural circuitry underlying the behavioral and metabolic effects of MBH leucine sensing.

Results from these studies have also begun to characterize the intra- and intercellular signaling networks engaged by MBH leucine sensing. Refeeding leucine rich food after a fast, and MBH leucine itself, each rapidly elevate S6K1 activity selectively within the MBH, but not in either PVN or lateral hypothalamic (LH) nuclei. These data suggest that MBH leucine's ability to drive S6K1 activation in the MBH may determine leucine's feeding inhibitory actions. Consistent with this suggestion, adenovirally mediated overexpression of S6K1 within the MBH decreased food intake and reduced body weight gain, and, similar to the effects of MBH leucine, these reductions in feeding were due to a selective reduction in meal size without a change in meal frequency. In contrast, downregulation of S6K1 activity by adenoviral MBH expression of a dominant negative form of the kinase promoted weight gain and increased meal size without increasing meal number [11].

Refeeding after a fast and MBH leucine administration also activate MBH Erk ½ signaling, implicated in multiple feeding neurocircuits (e.g. [12]). Pharmacological Erk ½ inhibition within the MBH rapidly stimulates food intake by selectively increasing meal size. Furthermore, pharmacological inhibition of Erk ½, at subthreshold doses of ERK antagonists that have no effect when administered alone, are able to block the feeding inhibitory actions on MBH leucine. These data support Erk1/2 phospohrylation as an alternative critical signaling mechanism linking MBH leucine sensing to the control of meal size. Furthermore, possible biochemical pathways underlying the ability of leucine to drive both S6K1 and ERK phsophorylation remain to be identified.

From an extracellular perspective, MBH leucine induces robust and selective expression of c-fos, a marker of neuronal activation, in multiple forebrain and hindbrain sites, including the ARC, PVN and the brainstem NTS, but not in lateral, supraoptic, or dorsomedial hypothalamic nuclei. Among leucine-activated ARC cells is a population of POMC-expressing neurons, suggesting that leucine sensing stimulates POMC ARC neurons. Consistent with this suggestion, neurophysiological evaluation of hypothalamic slices in vitro revealed that bath application of leucine rapidly stimulated neurophysiological spike activity in identified POMC neurons. MBH leucine also promoted c-fos expression in neurochemically defined oxytocinergic (OXY) neurons within the PVN, supporting an ARC POMC - PVN OXY link, activated by MBH leucine sensing. As oxytocin has been implicated in the inhibition of food intake, these data raise the possibility that PVN OXY neurons mediate the feeding inhibitory effects of MBH leucine. PVN oxytocinergic neurons project in part to the caudal brainstem, where oxytocin immunoreactive fibers have been localized to the DVC, at the level of the AP, particularly within the NTS. The NTS in this AP-spanning region has been well characterized as a terminus for peripheral neural gastrointestinal meal-related signals important in the negative feedback control of food intake and meal size [13]. Administration of oxytocin receptor antagonists restricted to the fourth ventricle, adjacent to the DVC, robustly and rapidly stimulates food intake and meal size in mice and rats [14]. Furthermore, administration of doses of oxytocin receptor antagonists that had no effect on feeding when administered alone blocked the ability of MBH leucine to reduce meal size [1]. Thus, MBH leucine sensing engages an ARC- PVN-NTS circuit via S6K1, ERK and oxytocin to control food intake and body weight gain.

BRAINSTEM LEUCINE SENSING IN THE CONTROL OF ENERGY BALANCE

The caudal brainstem DVC is an important site in the neural network engaging multiple effectors of energy balance, including food intake, glucose homeostasis, and thermogenesis. In particular, sensory neurons within the NTS of the DVC mediate the feeding behavioral and metabolic effects of gut nutrient stimulation, gut hormones, the adiposity hormone leptin, and descending hypothalamic inputs from neurons expressing orexigenic and anoreixgenic neuropeptides (e.g. [15]). These neurons are adjacent to the AP, a circumventricular organ with a specialized, fenestrated capillary system that enhances neuronal access to blood borne factors, presenting the additional possibility of direct nutrient sensing by NTS neurons. This possibility is supported by prior studies indicating that refeeding after a fast rapidly activates not only NTS c-fos expression, but also S6K1 and ERK ½ activation within NTS cells. Consequently, Blouet et al. [16] have recently shown that NTS neurons are directly sensitive to local injections of leucine. In fasted rats primed to eat, direct NTS injections of physiological doses of leucine rapidly and potently reduced food intake and body weight gain [16]. Within the first several hours post-injection, these reductions in feeding were solely due to reduced meal size, and were followed by reductions in both meal size and meal number beginning 6 h after leucine injection. In contrast, the non-ketogenic amino acid l-valine had no effect on feeding, demonstrating amino acid specificity for leucine nutrient sensing in the NTS, similar to what was found following MBH injection.

NTS leucine also rapidly and potently activated the mTOR pathway, increasing phosphorylation of S6K1 and ribosomal S6 protein, a downstream effector of S6K1. Conversely, direct parenchymal NTS application of the mTOR inhibitor rapamycin stimulated immediate feeding by increasing meal size, supporting a role for endogenous NTS leucine signaling through mTOR-S6K1 in the control of food intake and body weight. Accordingly, NTS application of subthreshold rapamycin doses that had no effect on feeding when administered alone completely blocked the feeding, meal size and body weight effects of NTS leucine. Furthermore, constitutive adenoviral overexpression of active S6K in the

NTS reduced food intake, body weight gain, and adiposity, and the reduction in food intake was solely due to smaller meal size, similarly to what had been shown following MBH S6K overexpression. These data reveal that there are multiple, parallel, and perhaps redundant roles for leucine sensing in the negative feedback control of food intake, meal size and body weight, specifically at forebrain and hindbrain regions that abut circumventricular organs.

Immunohistochemical evaluation of the brainstem following NTS leucine injections identified S6K activation in 3 neurochemically distinct populations: those expressing dopamine beta-hydrolxylase, tyrosine hydroxylase, and the anorexigenic peptide precursor POMC. This latter finding suggested that NTS leucine might engage brainstem melanocortin signaling via POMC NTS neuronal release of melanocortins onto brainstem MC3/4 receptors, implicated in the brainstem control of meal size [17] [18]. Consistent with this idea, NTS injection of the MC3/4 antagonist SHU 9119, at doses that had no effect on food intake when administered alone, blocked the ability of NTS leucine to reduce food intake and meal size.

As the feeding inhibitory effects of brainstem melanocortins are mediated in part by the Erk ½ signaling pathway [17], subsequent studies evaluated the role of ERK ½ in the ability of brainstem leucine to reduce food intake and meal size. NTS injection of ERK 1/2 inhibitors alone rapidly increased food intake and meal size, but this effect dissipated within a few hours after antagonist administration, without any longer term effect on body weight gain. Furthermore, NTS injections of subthreshold doses of ERK ½ inhibitors, that had no effect on feeding when administered alone, blocked the ability of NTS leucine to reduce food intake and meal size. Thus, NTS leucine sensing mechanisms important in the control of food intake and body weight share several important features in common with MBH leucine sensing: both induce reductions in food intake and body weight gain by selective reductions in meal size, both engage intracellular pathways involving mTOR-S6K and ERK ½ activation, and both appear to be mediated by activation of melanocortinergic signaling.

SUMMARY

Taken together, these recent advances identify neuroanatomicaly distributed nodes in a circuit involving at least 2 primary leucine sensing sites adjacent to circumventriular organs, with preferential access to blood borne nutrients: the MBH and the DVC. Activation of these leucine sensing sites engages multiple determinants of energy balance through the mTOR-S6K and Erk1/2 signaling pathways, including glucose homeostasis, food intake, and adiposity. These findings do not exclude the possibility that other important leucine sensing sites may be identified. More importantly, however, the present results raise the question of how these individual, distributed leucine sensing mechanisms act in concert to determine overall energy balance in response to endogenous dietary fluctuations in leucine availability, occurring throughout the cerebral ventricular circulation.

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REFERENCES CITED

1. Blouet C, Jo YH, Li X, Schwartz GJ. Mediobasal hypothalamic leucine sensing regulates food intake through activation of a hypothalamus-brainstem circuit. J Neurosci. 2009; 29:8302–8311. [PubMed: 19571121]
2. Smith QR. The blood-brain barrier and the regulation of amino acid uptake and availability to brain. Adv Exp Med Biol. 1991; 291:55–71. [PubMed: 1927691]

3. Shigemitsu K, Tsujishita Y, Miyake H, Hidayat S, Tanaka N, et al. Structural requirement of leucine for activation of p70 S6 kinase. FEBS Lett. 1999; 447:303–306. [PubMed: 10214966]
4. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, et al. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature. 2004; 431:200–205. [PubMed: 15306821]
5. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab. 2009; 9:311–326. [PubMed: 19356713]
6. Zhang Y, Guo K, LeBlanc RE, Loh D, Schwartz GJ, et al. Increasing dietary leucine intake reduces diet-induced obesity and improves glucose and cholesterol metabolism in mice via multimechanisms. Diabetes. 2007; 56:1647–1654. [PubMed: 17360978]
7. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. Nat Neurosci. 2002; 5:566–572. [PubMed: 12021765]
8. Ono H, Pocai A, Wang Y, Sakoda H, Asano T, et al. Activation of hypothalamic S6 kinase mediates diet-induced hepatic insulin resistance in rats. J Clin Invest. 2008; 118:2959–2968. [PubMed: 18618016]
9. Su Y, Lam TK, He W, Pocai A, Bryan J, et al. Hypothalamic leucine metabolism regulates liver glucose production. Diabetes. 2012; 61:85–93. [PubMed: 22187376]
10. Cota D, Proulx K, Smith KA, Kozma SC, Thomas G, et al. Hypothalamic mTOR signaling regulates food intake. Science. 2006; 312:927–930. [PubMed: 16690869]
11. Blouet C, Ono H, Schwartz GJ. Mediobasal hypothalamic p70 S6 kinase 1 modulates the control of energy homeostasis. Cell Metab. 2008; 8:459–467. [PubMed: 19041762]
12. Berthoud HR, Sutton GM, Townsend RL, Patterson LM, Zheng H. Brainstem mechanisms integrating gut-derived satiety signals and descending forebrain information in the control of meal size. Physiol Behav. 2006; 89:517–524. [PubMed: 16996546]
13. Schwartz GJ. Brainstem integrative function in the central nervous system control of food intake. Forum Nutr. 2010; 63:141–151. [PubMed: 19955782]
14. Blevins JE, Schwartz MW, Baskin DG. Evidence that paraventricular nucleus oxytocin neurons link hypothalamic leptin action to caudal brain stem nuclei controlling meal size. Am J Physiol Regul Integr Comp Physiol. 2004; 287:R87–96. [PubMed: 15044184]
15. Grill HJ, Hayes MR. Hindbrain neurons as an essential hub in the neuroanatomically distributed control of energy balance. Cell Metab. 2012; 16:296–309. [PubMed: 22902836]
16. Blouet C, Schwartz GJ. Brainstem Nutrient Sensing in the Nucleus of the Solitary Tract Inhibits Feeding. Cell Metab. 2012
17. Sutton GM, Duos B, Patterson LM, Berthoud HR. Melanocortinergic modulation of cholecystokinin-induced suppression of feeding through extracellular signal-regulated kinase signaling in rat solitary nucleus. Endocrinology. 2005; 146:3739–3747. [PubMed: 15961554]
18. Azzara AV, Sokolnicki JP, Schwartz GJ. Central melanocortin receptor agonist reduces spontaneous and scheduled meal size but does not augment duodenal preload-induced feeding inhibition. Physiol Behav. 2002; 77:411–416. [PubMed: 12419417]

KEY POINTS

- Recent advances identify neuroanatomicaly distributed nodes in a circuit involving at least 2 primary leucine sensing sites adjacent to circumventriular organs, with preferential access to blood borne nutrients: the MBH and the DVC.
- Activation of these leucine sensing sites engages multiple determinants of energy balance through the mTOR-S6K and Erk1/2 signaling pathways, including glucose homeostasis, food intake, and adiposity.