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MEMRI detects neuronal activity and connectivity in hypothalamic neural circuit responding to leptin

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ABSTRACT

Hypothalamus plays the central role in regulating energy homeostasis. To understand the hypothalamic neurocircuit in responding to leptin, Manganese-Enhanced MRI (MEMRI) was applied. Highly elevated signal could be mapped in major nuclei of the leptin signaling pathway, including the arcuate nucleus (ARC), paraventricular nucleus (PVN), ventromedial hypothalamus (VMH) and dorsomedial hypothalamus (DMH) in fasted mice and the enhancement was reduced by leptin administration. However, whether changes in MEMRI signal reflect Ca^{2+} channel activity, neuronal activation or connectivity in the leptin signaling pathway are not clear. By blocking L-type Ca^{2+} channels, the signal enhancement in the ARC, PVN and DMH, but not VMH, was reduced. By disrupting microtubule with colchicine, signal enhancement of the secondary neural areas like DMH and PVN was delayed which is consistent with the known projection density from ARC into these regions. Finally, strong correlation between c-fos expression and MEMRI signal increase rate was observed in the ARC, VMH and DMH. Together, we provide experimental evidence that MEMRI signal could represent activity and connectivity in certain hypothalamic nuclei and hence may be used for mapping activated neuronal pathway in vivo. This understanding would facilitate the application of MEMRI for evaluation of hypothalamic dysfunction in metabolic diseases.

Introduction

Obesity is caused by imbalance of energy homeostasis which involves food intake, energy storage and expenditure. A mechanism of the energy regulation is the "adiposity negative feedback" model with leptin, a 16 kDa protein secreted by white adipose tissue, as the main circulating signal in proportion to the body fat (Ahima et al., 2000; Elmquist et al., 1998; Frederich et al., 1995). Leptin level in plasma reduces during fasting and raises during re-feeding, suggesting energy regulation is a main function of leptin (Bjorbaek and Kahn, 2004). The main site of the leptin action is through binding to leptin receptors (Ob-Rb) in the hypothalamus (Bates and Myers, 2003) and brainstem which project to cortex and vagus nerve and hence results in change of food intake and energy expenditure (Morton et al., 2006). In obesity, central leptin resistance has been identified (Jung and Kim, 2013). Therefore, understand hypothalamic leptin signaling is crucial for understanding the central mechanism of obesity.

The first-order neurons responding to the leptin signals are anorexigenic proopiomelanocortin/cocaine and amphetamine-related transcript (POMC/CART) and orexigenic neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons in the arcuate nucleus (ARC) of the hypothalamus (Satoh et al., 1997). With leptin binding, POMC/CART neurons are activated while NPY/AgRP neurons are inhibited. Leptin signaling is further transduced through neuronal projections from ARC to other nuclei within and outside hypothalamus (Bouret et al., 2004). NPY neurons terminate in the paraventricular nucleus (PVN), dorsomedial nucleus (DMH) and lateral hypothalamic area (LH) in the hypothalamus, as well as to the nucleus of solitary tract (NTS) in the brainstem and anterior olfactory nucleus. NPY/AgRP fibers from LH further project to cerebral cortex that regulates hunger sensations (Sainsbury et al., 2002), therefore, it is also regarded as the "hunger centre" (Schwartz et al., 2000). Globally NPY/AgRP neurons function as restoration of energy homeostasis by stimulation of food intake during energy deprivation (Williams et al., 2001). POMC/CART neurons project in high density to PVN; moderate to DMH and LH; and only few to ventromedial hypothalamic nucleus (VMN). Outside the hypothalamus, POMC/CART also terminates at NTS, strongly supporting that leptin signaling is not restricted to hypothalamus only (Millington, 2007). NPY terminals and melanocortin receptors are also found in AgRP/NPY and POMC neurons themselves, suggesting autoregulation between NPY/AgRP and POMC/CART neurons. These

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anorexigenic and orexigenic neurons interact with other projected nuclei to regulate the food intake and energy expenditure (Sainsbury et al., 2002). The summery of leptin signaling neural network in the hypothalamus is illustrated in Fig. 1.

Being deep inside the brain, limited methods have been applied to investigate hypothalamic leptin signaling neural pathway from single unit to the whole system. Direct microinjections of leptin into ARC, VMH and LH lead to the decrease of food intake and body weight in order as following: ARC > VMH=LH with ARC being determined as the primary site of satiety effect of leptin (Satoh et al., 1997). Electrophysiology recording on POMC and NPY neurons confirmed the depolarization of POMC and hyperpolarization of NPY neurons by leptin (Cowley et al., 2001). C-fos immunostaining was used to understand the effects of neurotransmitters (α-MSH, AgRP) under fasting and refeeding conditions, which shows activation of subsets of neurons in PVN and DMH due to anorexic effect of α-MSH (Singru et al., 2007). However, how the higher order neurons interact among each other is largely unknown. Recently in vivo Ca^{2+} imaging was applied to measure responses of γ-aminobutyric acidergic (GABAergic) neurons in LH (Jennings et al., 2015), but it is difficult to image all nuclei due to the restrictive field-of-view.

Functional MRI has been used to detect the hypothalamic response to appetite-modulating signals such as glucose, diets and peptides (Min et al., 2011). Attenuated BOLD response to glucose was detected in obese rats (Chen et al., 2007). FMRI of hypothalamus in mice is difficult due to sensitivity and resolution limitations. Using Diffusion-Weighted Imaging (DWI), diffusion coefficient change in ARC, VMH and DMH under fasting was used to implicate hypothalamic activation (Lizarbe et al., 2013b). However, diffusion is dependent on variety of factors therefore inferring neural activation is highly arguable. ^{1}H MRS is able to measure metabolic profile of the hypothalamus and shows higher GABA and lower taurine concentrations compared to other cerebral structures (Lei et al., 2010). ^{13}C and ^{1}H high resolution magic angle spinning spectroscopy (HRMAS) revealed increase of GABAergic neurotransmission and glycolysis as result of overnight fasting (Violante et al., 2009). However, in vivo MRS cannot resolve nuclei in hypothalamus while HRMAS is limited to ex vivo samples.

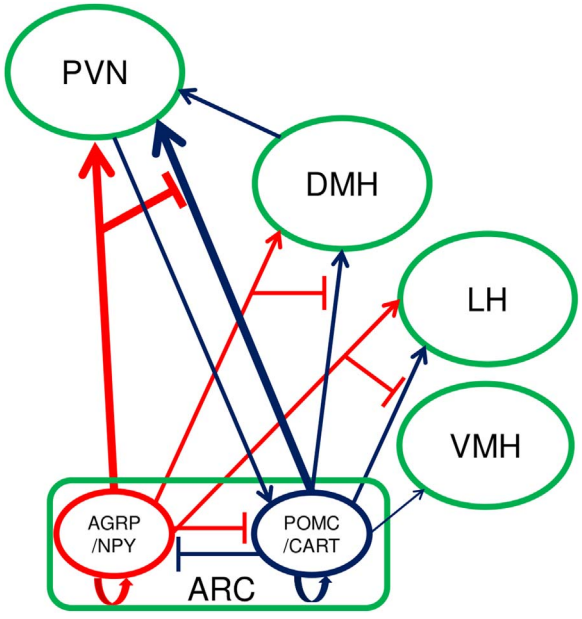

Fig. 1. Leptin signaling AgRP/NPY and POMC/CART projections in the hypothalamus. Blue color represents POMC/CART neuronal projections, red color represents AgRP/NPY neuronal projections, thickness and arrow of the lines represent density and direction of projections. The nuclei in the pathway include arcuate nucleus (ARC), paraventricular nucleus (PVN), dorsomedial nucleus (DMH), ventromedial hypothalamic nucleus (VMN) and lateral hypothalamic area (LH).

Manganese-Enhanced MRI (MEMRI) has been successfully applied to study activity of the hypothalamus. Mn^{2+} has structure similar to Ca^{2+} and can enter active neural cells through voltage-gated Ca^{2+} channels. After being taken up in neurons, Mn^{2+} can be transported along axonal tracts via microtubule, released at synapse and taken up into the postsynaptic neurons (Chuang and Koretsky, 2006; Silva et al., 2004). Mn^{2+} has limited permeability across BBB via transporters such as transferrin receptor (TfR) (Bornhorst et al., 2012) and the divalent metal transporter-1 (DMT-1)(Aschner, 2006). The majority of Mn^{2+} has been shown to enter brain through choroid plexus and circumventricular organs due to specialized ependymal cells in that area (Lee et al., 2005). Similarly medium eminence (ME), which is located next to the ARC, allows axon terminals to reach outside of BBB (Rodriguez et al., 2010). As a result, Mn^{2+} can enter ARC through ME and then propagate to other nuclei in the hypothalamus via axonal transport, passive diffusion or other mechanisms (Parkinson et al., 2009). Pioneer works by Bell's group showed that MEMRI could pick up change in ARC, VMH, PVN and PeVH of hypothalamus under physiological manipulation like fasting without BBB disruption (Kuo et al., 2006), and administration of orexigenic peptides such as ghrelin resulted in increased intensity in ARC, PVN and VMH while anorexigenic hormone peptide YY_{3-36} led to reduced intensity (Kuo et al., 2007). Further studies have been done to investigate hypothalamic functions related to feeding behavior, response to hormonal (such as oxyntomodulin and glucagon-like peptide-1 (GLP-1) (Chaudhri et al., 2006) or pancreatic polypeptide (Hankir et al., 2011; Parkinson et al., 2009)) and physiological manipulations (Lizarbe et al., 2013a). MEMRI has also been applied to transgenic model of leptin deficiency and shown increased activity in ob/ob mice supported by increases in neuronal oxidative metabolism and glutamatergic neurotransmission (Delgado et al., 2011) with different hypothalamic response to diet adjustments (Anastasovska et al., 2012; Just and Gruetter, 2011).

Since Mn^{2+} uptake into postsynaptic neurons is also activity dependent, this could enable the detection of active neural pathway by following Mn^{2+} transport through the neural system (Chuang et al., 2009). Therefore MEMRI may allow assessing neural circuit activity from the primary area, ARC, to the secondary areas like DMH, PVN, etc, in the hypothalamus. However, due multiple factors influencing Mn^{2+} uptake and transport in the cells, to what extent can MEMRI signal represent neuronal activity and connectivity in the hypothalamus is still not clear. Here, we evaluated MEMRI for mapping leptin dependent neuronal pathway in hypothalamus and assessed the signal in relating to Ca^{2+} channel activity by blocking Ca^{2+} channel, axonal connectivity by microtubule disruption, and neuronal activation by c-fos immunofluorescence staining. The results show that MEMRI can provide valuable and unique information on hypothalamic neurocircuit activity and connectivity.

Materials and methods

Experimental design

Study 1: MEMRI of hypothalamic response to leptin

To determine whether MEMRI can detect leptin induced activity change in the hypothalamus, 0.01 mg/kg leptin (R & D Systems, USA) was injected as bolus via i.p into non-fasted (n=5) and fasted (n=5) mice after Mn^{2+} reached the blood stream. Phosphate buffered saline (PBS) was injected in another groups of non-fasted (n=6) and fasted (n=5) mice as vehicle controls. For Mn^{2+} injection, 62.2 mM $MnCl_2$ (Sigma-Aldrich, Singapore) in PBS was infused via i.v. with a dose of 62 mg/kg and an infusion rate of 0.2 ml/h by a syringe pump (Achema Pte Ltd, Singapore). To measure plasma leptin level, blood samples were taken from the tail vein from another sets of non-fasted (n=5) and fasted (n=5) mice. Mouse leptin level was measured using an ELISA Kit (EMD Millipore Corporation, USA).