Mapping CNS Response to Leptin by MEMRI

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Introduction

Energy homeostasis is regulated by a very complex central & peripheral nervous system through various signaling pathways, such as adiposity signaling and satiety signaling pathways [1]. Hypothalamus plays a central role in energy homeostasis – with Arcuate Nucleus (ARC) as the primary gateway projecting excitatory/inhibitory signals to other hypothalamic nuclei, which further project to cortical regions and brainstem, thus forming multiple neural circuits to regulate energy balance and behavior [2]. Leptin is one of the major adiposity signal which inhibits food intake and increases energy expenditure through interactions with its specific receptors in the brain. It is an important regulatory signaling pathway that gets disrupted in obesity. However, the underlying neural circuit and activity in different nuclei under leptin resistance is still not clear and is an area of active research [3].

To understand the activity of hypothalamic nuclei in responding to leptin signal, we explored the use of manganese enhanced MRI (MEMRI). Mn^{2+}, being Ca^{2+} analog, can get into activated neurons thru voltage-gated Ca^{2+} channel. Although Mn^{2+} does not cross BBB easily, ARC in hypothalamus is next to Median Eminence which lacks tight junction in capillaries – placing it outside effective BBB coverage and hence ARC neurons have direct access to circulating metabolic signals and chemicals, including leptin and Mn^{2+}. Together with its paramagnetic property in shortening T_1 relaxation time, Mn^{2+} makes activated nuclei visible by changing signal intensity (SI) in T_1-weighted MRI [4].

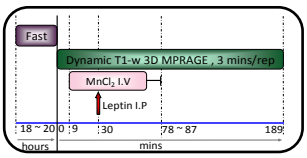

Fig. 1. Expt. Procedure Timeline

Materials and methods

All animal studies were approved by the local IACUC. Male C57BL/6 animals, (3 – 5 months old, body weight: 28 ~ 34 g) were used in this study. 4 groups of experiments were conducted: fasted (n=3), non-fasted (n=3), fasted injected with leptin (n=3) and non-fasted injected with leptin (n=2). Animals were collected from animal holding facility 20 hours prior to scanning and acclimatized in a holding cabinet adjacent to the MRI facility. All scans started between 4:00 pm ~ 4:30 pm to avoid diurnal variations in brain activity. For the fasted groups, animals were fasted for 18 ~ 20 hours before MRI scan (Fig. 1). MRI was acquired on a 9.4T/31-cm magnet interfaced to a Varian console. Dynamic T1-w 3D MP-RAGE images of 75×75×400 μm^3 resolution were acquired for about 3 hr, with each time frame taking 3 mins. SNR of 3 initial frames were averaged to obtain baseline SNR. $MnCl_2$ (5ml/Kg, 62.3mM) was infused via i.v. at an infusion rate of 0.2ml/hr. Leptin was injected as a bolus via i.p. (10μg/ g) at 21 min after Mn^{2+} infusion (Fig. 1). SNR of all the frames were normalized to the baseline. ROIs were manually drawn in ARC, Ventromedial Hypothalamic Nucleus (VMH), Paraventricular Nucleus (PVN) and Dorsomedial Hypothalamic Nucleus (DMH) based on the Paxinos Mouse Brain Atlas [5]. Mean time course in the ROIs were obtained, and the mean SI of the last 10 data points was considered as the plateau value. The time required to reach the plateau (time-to-plateau), the uptake rate defined as slope of the signal enhancement from 15% to 85% of plateau, and enhancement beginning time defined as the intersection of the uptake slope and the baseline were estimated. Two-tailed unpaired t-test was used to compare the groups and $p<0.05$ was regarded as significant.

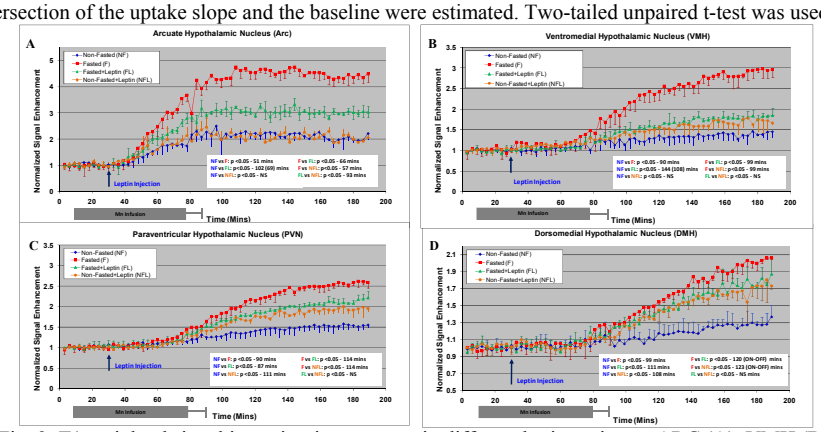

Fig. 2. T1-weighted signal intensity time-courses in different brain regions – ARC (A), VMH (B), PVN (C), DMH (D) of Fasted, Non-Fasted, Fasted+Leptin and Non-Fasted+Leptin animals.

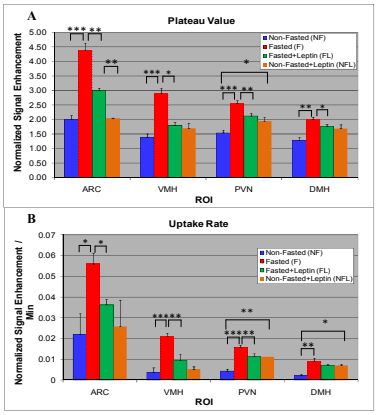

Fig. 3. Estimated (A) Plateau value; (B) Uptake rate

Results

High resolution, and good sensitivity and stability were achieved with the 3D MPRAGE. Mean SI time course of the ROIs and the time when the SI started to become significantly different are shown in Fig. 2. It was observed that the SI in ARC shows the highest enhancement among the hypothalamic nuclei studied and the enhancement saturates around the time when Mn^{2+} infusion completed, which was between 80 ~ 90 mins after the beginning of scan. Signal enhancement in secondary nuclei, such as VMH, PVN and DMH, was observed to be significantly lower compared to ARC, with an average delay of 20 ~ 30 mins before detectable SI increase. The signal after injection of leptin was suppressed significantly in most nuclei in fasted animals. In non-fasted animals, however, leptin injection increased SI in some nuclei such as PVN. Significant difference was observed in plateau value and uptake rate among groups (Fig. 3), where as there was no significant difference observed in time-to-plateau or enhancement beginning time with leptin injection.

Discussion

We showed that enhancement or suppression of SI in hypothalamic nuclei by leptin can be detected by MEMRI with individual nuclei having different responses to leptin under fasted and non-fasted condition. For example, leptin reduced SI in ARC in fasted but did not change in non-fasted animals, while leptin enhanced SI in other nuclei in non-fasted animals. Interpretation of MEMRI signal kinetics is complex as this technique cannot discriminate between the excitation and inhibition in different populations of neurons in the same region. Change in SI therefore indicate ensemble of activation obtained from excitatory and inhibitory activities. No change in SI may therefore represent either lack of activation, or balanced net excitation and inhibition. Since ARC contains two major neuronal subpopulations – orexigenic neurons (NPY/AgRP) which are inhibited by leptin and anorexigenic neurons (POMC/CART) which are activated by leptin, the unchanged SI in non-fasted condition suggests a balance of activity of both subpopulations in ARC. In other nuclei, which contain only excitatory neurons for leptin, they were activated in non-fasted condition. In fasted animal, leptin inhibited the NPY/AgRP neurons and activated the POMC/CART neurons in ARC and signal indicates that there is a net inhibition. The activation in VMH in fasted animals is contradictory to older literatures as it has been considered as the satiety centre [2] but recent studies suggested that glucose sensing neurons in VMH gets stimulated by increased level of ghrelin due to fasting [6,7].

In conclusion, this technique can be applied to study hypothalamic function and its response to different metabolic signals. This will facilitate our understanding of hypothalamic dysfunction under leptin resistance using models eg, high-fat diet, or db/db transgenic animals.

References:

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