Manganese-Enhanced MRI of Appetite Centres in ob/ob Mice

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Introduction

Objective measurement of neuronal activity in relation to appetite control currently uses methods that are either highly invasive and/or fatal, such as immunohistochemistry for the early-expressed neuronal activation protein c-Fos. To measure changes over time using such methods requires parallel study groups culled at the different times. A non-invasive strategy such as using Manganese-Enhanced MRI (MEMRI) to analyze the same individuals over a time course would 1) reduce the number of animals used, 2) reduce experimental variation, and 3) possibly allow the direct study of humans.

We recently determined that MEMRI (with Mn2+ administered by intravenous infusion) could, in terms of magnitude at several appetite centres in the brain, differentiate between the neuronal activities of mice that had been fed normally or fasted overnight. The aim of this study was to extend the above feeding-fasting experiment to leptin-deficient (ob/ob) mutant mice. The ob/ob phenotype includes obesity, hyperinsulinaemia, hyperlipidaemia and defective thermoregulation. This mouse model is used extensively to probe the mechanisms of mammalian hunger control, so it was essential to determine whether MEMRI could perform well in this situation.

Materials and Methods

All experiments complied with the Animal (Scientific Procedures) Act 1986 (UK). Mice were 11-15 wk old homozygous C57BL/6OlaHsd-Lepobob males (Harlan, UK) referred to here as ob. Isoflurane anaesthetised mice were implanted with a tail vein cannula and placed in a 25 mm internal diameter quadrature head coil (Magnetic Resonance Laboratories, Oxford, UK). The cannulated mouse, coil and a saline phantom were placed at the centre of a 9.4 T horizontal bore MR scanner (Varian, Palo Alto, CA, USA). Sixty-six image stacks of the brain were acquired consecutively over a period of just over 2 hours (1 m 57 s per acquisition) using a spin-echo multi-slice T1-weighted sequence with TR=600 ms, TE=10 ms, a 256x192 voxel matrix (zero-filled to 256x256), 25 mm × 25 mm field of view, single averages, and 10 transverse slices of 1 mm thickness each. After three baseline acquisitions, 62.3 mM MnCl2 was infused via the cannula by a syringe pump (PHD 2000, Harvard Apparatus, MA, USA) for around 40 mins (exact time proportional to body weight) at a rate of 0.2ml/h. The signal intensity (SI) of different brain structures was measured by drawing regions of interest (ROI) with the image processing software Image J (version 1.33, NIH, USA). Corrections were made for machine, infusion and inter-animal variation by normalization to phantom SI, SI of 4th ventricle/choroids plexus boundary, and SI of the baseline scans respectively.

Results

Mice in groups of six were either fasted for 12-18 hrs overnight or fed normally before being processed and imaged by MEMRI. Enhancement (% SI increase over baseline) reached a steady-state during final 15 image stacks as reported previously.

The results of the MEMRI analysis are presented in the Figure. The first feature to note is that fasting state does not regulate the enhancement of the AP in ob mice, providing evidence that MEMRI behaves similarly in the ob and wild-type environments.

No significant differences in enhancement were found between fed and fasted groups of ob. When fed and fasted groups were combined, despite the inevitable increase in variance, significant differences in enhancement were found between lean and ob groups for most brain regions (Pe, VMN and PVN p<0.001, AP p<0.05). In all cases apart from the AP, enhancement was higher in ob mice. An interesting comparison is that between fasted lean and fed ob. This test considers whether the enhancement due to fasting (an acute, short-term situation) is of a similar magnitude to that generated as a result of starvation (a chronic, long-term situation that ob mice represent). Again, enhancement was higher in ob mice in all brain regions apart from the AP where it was lower. However, these differences were only significant in the cases of the Pe and PVN (p<0.05).

Discussion

In this study, as measured by MEMRI, no significant differences were found in the neuronal activity of appetite-regulating brain regions between fed and fasted groups of ob mice. This is probably a consequence of the acute feeding state effect being saturated by the chronic starvation effect. However, inconsiderate of feeding state, ob mice were found to have significantly higher levels of activity than lean in the Pe, VMN and PVN. Even fasted lean mice had lower activities in their appetite VMN. These results are expected since ob mice are innately starved, so the activity of their appetite centers should be higher than that of lean mice.

There is some agreement between these results and those from c-Fos immunohistochemistry, which suggest that feeding state has no significant effect on ob neuronal activity. However, the c-Fos data indicates that ob mice merely have moderately raised levels of activity in their appetite centres compared to lean. Thus MEMRI and c-Fos results are consistent with respect to acute effects, but diverge with respect to chronic effects. This discrepancy needs to be resolved, and may reveal important information about the relationship between c-Fos levels and neuronal activity, which currently is not known beyond a few hours.

In conclusion, in vivo MEMRI confirms previous in vitro c-Fos results for acute fasting in the ob environment, though there are discrepancies with results of chronic states.

References

2. Kuo, Y.-T., Herlihy, A. H., So, P-W., Bell, J.D. in ISMRM Thirteenth Scientific Meeting and Exhibition (Miami Beach Convention Center, Miami Beach, Florida, USA, 2005).