Imaging Appetite in vivo with Manganese-enhanced MRI (MEMRI)

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Introduction: Obesity is currently a major health problem across the world. The mechanisms underlying the development and maintenance of obesity are not fully understood, although patterns of eating are undoubtedly altered in obesity. The discovery of appetite-regulating neuropeptides is providing new insights into the mechanisms associated with appetite and satiety and may lead to new pharmacological targets for treating obesity. Although c-fos expression in vitro has helped to identify some of the regions of the brain activated during appetite and satiety, repeated examination cannot be performed on the same animal and temporal responses to stimuli are likely to require studies involving extremely large numbers of animals. Neuroimaging provides in vivo information about the regions of neuronal activity associated with behavior and stimuli in animals and man. Manganese-enhanced MRI (MEMRI) has been proposed as a potential method to delineate neuronal connectivity and to assess brain structures in experiment animals (1, 2). In this study we have extended the use of MEMRI to determine the neuronal activations associated with appetite in a murine model, using ghrelin (the so-called "hunger hormone") as a test bed. The results from this study suggest that this methodology can be used to establish the site, degree and timing of neuronal activity associated appetite and satiety.

Methods: Normal male C57BL/6 mice (16 – 24 weeks old) were anaesthetized with isoflurane-oxygen mixture and scanned with spin-echo multi-slice sequences on a 9.4T horizontal-bore MR scanner (Varian, USA) before and after intravenous (IV) administration of 100 mM $MnCl_2$ ($MnCl_2 \cdot 4H_2O$) from tail vein and intraperitoneal (IP) administration of ghrelin (0.06 and 0.3 nmol/g body weight; N = 5 and 4, respectively) or their vehicle solution (PBS; N = 4). The dose for IV $MnCl_2$ injection was 5 ml/kg body weight. Spin-echo multi-slice T1-weighted sequence was carried out with following scanning parameters; repetition time (TR) = 600 msec, echo time (TE) = 18 msec, matrix = 256 x 192, field of view (FOV) = 25 mm x 25 mm, number of averaging = 1, number of slices = 10 and slice thickness = 1 mm. The scanning time for each acquisition at a time point is 1 min 57 sec. After 3 baseline scans, manual injection of ghrelin or PBS was performed through IP catheter and $MnCl_2$ administration by a syringe injection pump (PHD 2000, Harvard Apparatus, MA) at injection rate of 0.2 ml/hr starting as well. This was followed by continuous 63 scans which last for about 2 h. Follow-up scans at 3h, 4h, 5h and 6h for each subject were also performed. The rectal temperature was monitored and maintained at 35 – 35.5 ^oC by a heating system (SA Instruments, Inc, NY). The signal intensities (SI) of arcuate hypothalamic nucleus (Arc), periventricular hypothalamic nucleus (Pe), lateral hypothtalamus (LH), paraventricular hypothalamic nucleus (PVN), anterior pituitary gland (AP), 4th ventricle and phantom filled with normal saline were measured by drawing the regions of interest (ROI) on corresponding areas with a imaging processing software (Image J 1.3.1, NIH, US). The normalized SI (signal intensity of target area/signal intensity of water phantom) at each time point was obtained. The time signal intensity curve was created with computer software (Prism 4, GraphPad Software, CA). ANOVA was used to compare the difference between different groups. A $P < 0.05$ was considered statistically significant.

Results & Discussions: With IV infusion of $MnCl_2$, the SI for structures outside the blood-brain barrier (BBB) (Fig 1) increased significantly as early as 6 min post starting infusion, and reached peak enhancement at about 40 – 50 min. For those inside the BBB (Fig 2), the SI increased gradually reaching a plateau around 70 min post initiation of infusion. The time-course curves at different brain regions showed different responses to IP ghrelin injection. Administration of ghrelin led to a significant modulation in the relative intensity of the time-curve of the $MnCl_2$ enhancement, which appear to be region specific. In the Arc (Fig 1) and Pe, significantly higher SI was observed after $MnCl_2$ infusion in the presence of ghrelin at both doses compared to PBS ($P < 0.001$). However, no significant difference ($P > 0.05$) was observed between different doses of ghrelin. In the LH (Fig 2), a dose response modulation of the Mn^{2+} associated increase in SI ($P < 0.001$) observed. Furthermore, this dose-dependent time-course response extended to at least 6h post-administration (Fig 3). In PVN, mice injected with high-dose ghrelin showed higher SI than the two other groups ($P < 0.001$). No significant difference in SI was observed in AP and in the 4th ventricle in the presence of ghrelin at either dose ($P > 0.05$). The figure 4 demonstrates the enhancement at Arc and LH after administrations of $MnCl_2$ and ghrelin. Mn^{2+} has been previously shown to get into cells via voltage-gated Ca^{2+} channel, and therefore has been utilised as a potential marker of neuronal activation. In this study we have shown that Mn^{2+} enhancement in the brain can be modulated by the gut-peptide ghrelin in a region specific manner, similar to those previously reported by c-fos in vitro. Interestingly, the Arc, which is known to be part of the neurons that regulate appetite, showed a different enhancement profile to other regions also associated with appetite, including the hypothalamus. This appears to arise from the fact that the Arc is also outside the full coverage of BBB, allowing $MnCl_2$ to more readily get into it and adjacent neurons via blood stream. However, despite these anatomical differences, significant modulation was still observed following the administration of ghrelin.

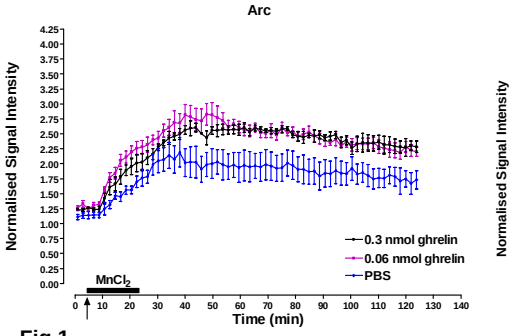

Fig 1

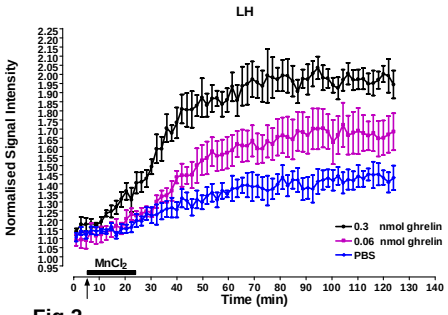

Fig 2

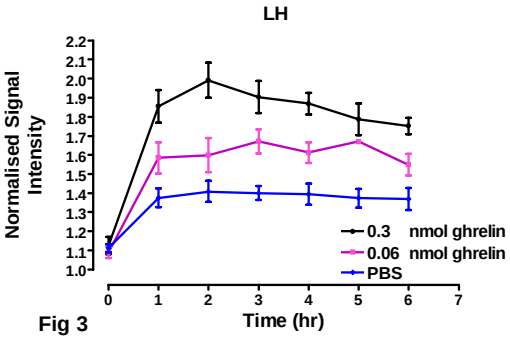

Fig 3

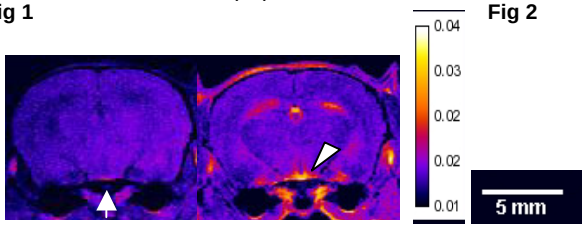

Fig 4. The left figure shows transverse T1W image before administration of ghrelin or MnCl2. The right figure shows significant enhancement over Arc (arrow) and LH (arrowhead) 1 hour after IP ghrelin and IV MnCl2 administrations.

Conclusion: We have shown that systemic administration of $MnCl_2$, without compromising BBB integrity, can be utilised to delineate the neuronal activations induced by ghrelin. In addition, the dose-dependent responses observed in a site-specific manner suggest that this technique might be an invaluable tool to evaluate in vivo hypothalamic activations associated with appetite and satiety under physiological conditions.

References: 1. Morita, H. et al. Neurosci Lett 326:101-104, 2002
2. Aoki, I. et al. Magn Reson Med 48:927-933, 2002
3. Saleem KS. et al. Neuron 34:685-700, 2002