Anorexigenic Gut Hormone Oxyntomodulin Modulates Manganese-enhanced MRI Signal In The Mouse Hypothalamus

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Introduction: Obesity is a major cause of morbidity and mortality worldwide. Currently available therapies are of limited effectiveness and have significant side effects. The need for more effective treatments has imparted impetus to efforts directed at understanding the mechanisms underlying energy balance. Gut hormones are important regulators of the complex neuroendocrine interactions that govern the regulation of appetite and food intake [1]. Gut hormones released into the circulation after a meal modulate neuronal circuits within important areas of the central nervous system, notably the arcuate (ARC) and paraventricular (PVN) nuclei of the hypothalamus. Manganese-enhanced MRI (MEMRI) is increasingly being used to investigate neuronal activity *in vivo*. Here, we investigate the modulation of MEMRI signal intensity (SI) in various regions of the mouse hypothalamus in response to injections of the gut hormone oxyntomodulin (OXM). OXM is known to reduce food intake when administered to rodents and humans [2,3].

Methods: *Animals:* C57BL/6 mice (16-24 wks old) were used. General anaesthesia was induced with 1.5% isoflurane in O_2 and maintained at 1% isoflurane in O_2. *MRI:* A 9.4T horizontal bore scanner (Varian, Palo Alto, CA) was used in conjunction with a quadrature mouse head coil with a 25mm internal diameter. A 100 mM solution of $MnCl_2$ (buffered in 100 mM Bicine and NaOH to give a pH of 7.4) was used. 5 µl/g body weight was administered intravenously via the tail vein at a rate of 0.2 ml/hr. Spin-echo T1-weighted images were obtained: TR/TE = 600/10 msec; matrix = 256 x 192, zero filled to 256 x 256; field of view = 25 x 25 mm; average = 1; slice thickness = 1 mm; 10 slices per acquisition; scanning time = 1 min 57 secs per acquisition. After three baseline acquisitions, infusion of $MnCl_2$ was begun and OXM or vehicle was given by intraperitoneal (ip) injection.

Experimental Groups: Four experimental groups were investigated: 1) mice that had access to standard chow *ad libitum* and were injected ip with vehicle ('fed controls'); 2) mice that had been fasted for 16 hours prior to scanning and were injected ip with vehicle ('fasted controls'); 3) & 4) mice that been fasted for 16 hours prior to scanning and were injected ip with OXM at doses of 900 nmol/kg and 5400 nmol/kg. These doses were selected based on feeding study data: 900 nmol/kg had been shown to reduce 1-hour food intake in fasted mice by 50% compared with controls, and 5400 nmol/kg reduced 1-hour food intake by 90%.

Image Analysis: Regions of interest (ROI) were defined based on hypothalamic nuclei known to be important in the regulation of feeding behaviour: the ARC, PVN, ventromedial hypothalamic nucleus (VMH) and the supraoptic nucleus (SON). SI was measured using analysis software (Image J, NIH) and normalised against a saline-filled phantom scanned with the mouse and expressed as a percentage of baseline SI. In order to quantify the differences between experimental groups, the mean of the SI at 59, 61 and 63 minutes was calculated for each group and this was then compared across groups. This time period was chosen as it corresponds to the earliest timepoint for which food intake data are available for the doses of OXM used.

Statistics: Statistical analysis was performed using the Kruskal-Wallis test with Dunn's post test.

Results: Figure 1 shows the regions of interest used for image analysis. Infusion of $MnCl_2$ caused an increase in SI in all ROI studied over time. Figure 2A shows the timecourse of SI changes in the ARC for each of the experimental groups. Figure 2B shows the mean SI of timepoints 59, 61 and 63 minutes for each experimental group (expressed as percentage change over baseline) in each of the ROI. OXM causes a dose-dependent modulation of MEMRI SI in both the ARC and the PVN. Fasting resulted in an increase in signal in the SON compared with fed controls, which was lost at both doses of OXM tested. OXM at a dose of 5400nmol/kg caused a non-significant trend towards an increase in signal in the VMH.

Conclusions: We have demonstrated a region-specific, dose-dependent modulation of MEMRI SI by the anorexigenic gut hormone OXM in key areas of the hypothalamus. In both the ARC and PVN, there is no statistical difference in normalised SI, expressed as a percentage of baseline, between non-fasted mice and fasted mice injected with 5400 nmol/kg of OXM. This is consistent with the dose-effect relationship observed for OXM in feeding studies and with the known pattern of c-Fos immunoreactivity induced by OXM [2]. The trend towards an increase in SI in the VMH in mice injected with 5400 nmol/kg OXM is a new finding. The VMH is not an area previously implicated in the actions of OXM, and the lack of difference between fed and feasted control mice suggest that the effect of OXM in this region is distinct from its effect of inducing satiety. MEMRI therefore constitutes a potentially useful tool for the investigation of the site, degree and timing of the effects of gut hormones on key central nervous system regions involved in control of food intake.

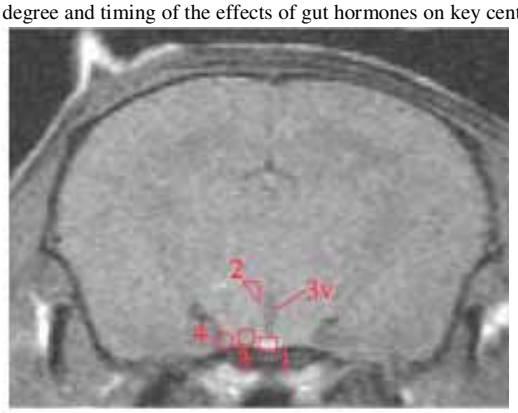

Figure 1 (above): Hypothalamic regions of interest used for image analysis. 3v: 3rd ventricle; 1: arcuate nucleus; 2: paraventricular nucleus; 3: ventromedial nucleus; 4: supraoptic nucleus.

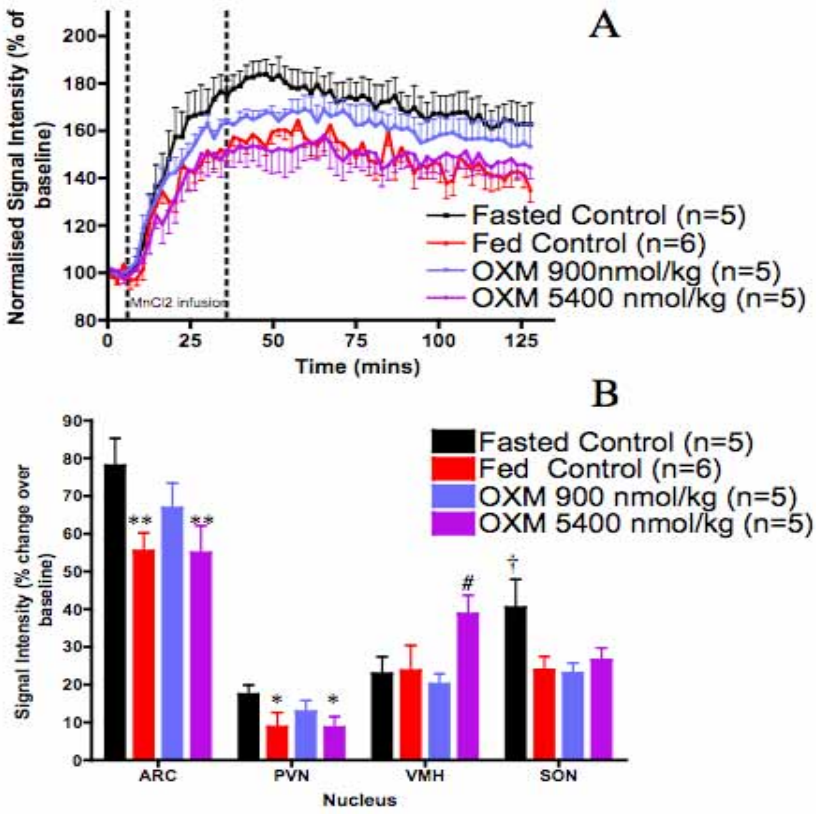

Figure 2 (right): A) Change in normalised signal intensity (expressed as a percentage of baseline) over time for the arcuate nucleus region of interest. B) Mean normalised signal intensity for the timepoints 59, 61 and 63 minutes for each region of interest (expressed as a percentage change over baseline). *P<0.05 compared with fasting control; **P<0.01 compared with fasting control; #P=0.062 compared with fasting control; †P<0.05 compared with other groups.

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[3] Cohen *et al.* (2003) *J Clin Endo Metab* 88:4696-4701.