Manganese-enhanced MRI Detects Distinct Patterns Of Neuronal Activation Following Anorexigenic Gut Hormone Administration

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Introduction: Obesity is responsible for 30,000 premature deaths in the United Kingdom annually and its increasing prevalence presages significant adverse public health and economic consequences worldwide. The control of appetite is complex and the limited success of discretional calorie restriction in achieving weight loss illustrates the powerful nature of the mechanisms driving feeding. Gut hormones are key physiological regulators of appetite with considerable therapeutic potential. Therapies based on glucagon-like peptide-1 (GLP-1) are at an advanced stage of development, but nausea has proved a troublesome side effect. Oxyntomodulin (OXM) is a related peptide, thought to act via the GLP-1 receptor [1;2], but which differs from GLP-1 in the scope of its biological activity and for which nausea is less problematical. Here, we use manganese-enhanced MRI (MEMRI) to compare the patterns of neuronal activity in key appetite-modulating regions of the mouse central nervous system (CNS) following systemic GLP-1 and OXM administration.

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) was used. 5 µl/g body weight was administered intravenously via the tail vein at a rate of 0.2 ml/hr. Spin-echo T_1-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, GLP-1 or vehicle was given by bolus 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 ('non-fasted controls'); 2) mice that had been fasted for 16 hours prior to scanning and were injected ip with vehicle ('fasted controls'); 3) mice that been fasted for 16 hours prior to scanning and were injected ip with 1400 nmol/kg OXM; 4) mice that been fasted for 16 hours prior to scanning and were injected ip with 900 nmol/kg GLP-1. These doses were selected as approximately bioequivalent, based on feeding study data: both doses reduced 1-hour food intake by approximately 50% after ip injection.

Image analysis: Regions of interest (ROI) were defined based on areas of the CNS known to be important in the regulation of feeding behaviour: the arcuate nucleus (ARC), paraventricular nucleus (PVN), ventromedial nucleus (VMH) and supraoptic nucleus (SON) of the hypothalamus, and the area postrema (AP) of the brainstem. Signal intensity (SI) was measured using analysis software (Image J, NIH), normalised against a saline-filled phantom scanned simultaneously with the mouse and expressed as a percentage of baseline SI.

Statistics: Generalised estimating equation curve analysis was used to compare the SI profile in each ROI across time and between treatment groups.

Results: Data are presented as mean ± SEM of three adjacent timepoints. Significant differences in SI were seen between non-fasted and fasted mice in the ARC, PVN, SON and AP. OXM administered on a fasted background resulted in SI profiles in the ARC, SON and AP that were significantly different to those of the fasted controls, but not different to those of the non-fasted controls. GLP-1, however, caused a significant change in SI in the PVN, VMH and AP (Figure 1 and Table 1).

Conclusions: The changes seen in SI in distinct regions of the CNS following gut hormone administration are consistent with the physiological role of these hormones as postprandial satiety signals. We have demonstrated that OXM and GLP-1 elicit distinct patterns of neuronal activation in the hypothalamus, as assessed by MEMRI. In addition, these data support a role for the brainstem in the mediation of the activities of both hormones, consistent with data obtained from studies of immediate-early gene activation and studies in animals that have undergone ablation of the vagal-brainstem-hypothalamus pathway [1;2;3]. Further studies, to correlate these distinct patterns of neuronal activation with differences in the biological properties of GLP-1 and OXM, would form a basis for the therapeutic exploitation of these hormones.

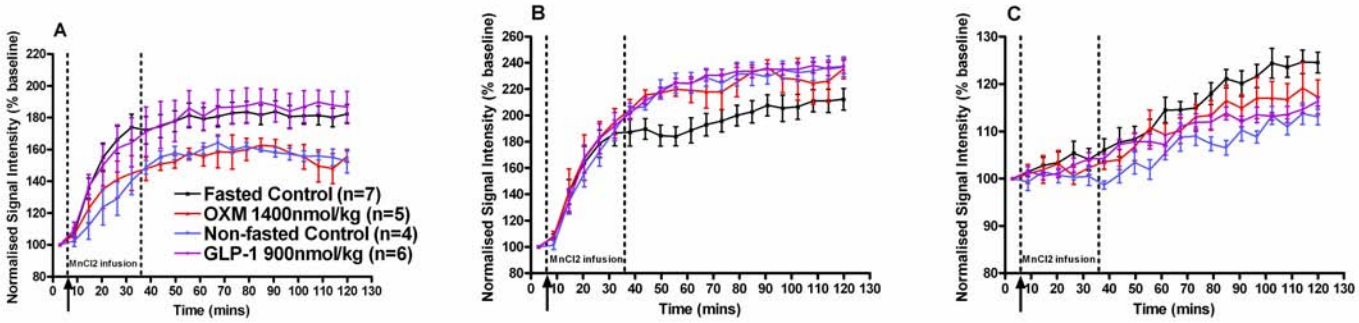

Figure 1: Change in normalised signal intensity (expressed as percentage of baseline) over time in the ROI corresponding to A) the arcuate nucleus; B) the area postrema; C) the paraventricular hypothalamic nucleus. Dotted lines represent mean period over which $MnCl_2$ was infused intravenously. Arrow indicates time of bolus intraperitoneal injection.

Comparison	Region of Interest				
	ARC	PVN	SON	VMH	AP
Fasted vs Non-fasted Controls	0.001	0.002	<0.001	NS	0.032
OXM vs Fasted Controls	0.002	NS	<0.001	NS	0.04
GLP-1 vs Fasted Controls	NS	0.022	NS	0.031	0.002

Table 1: P values (obtained by generalised estimating equation curve analysis) for key comparisons across treatment groups in the regions of interest considered.

References: [1] Dakin *et al.* (2004) *Endocrinology* 145:2687-2695
[2] Baggio *et al.* (2004) *Gastroenterology* 127:546-58
[3] Abbott *et al.* (2005) *Brain Res* 1044:127-31