Development and validation of a radioligand receptor binding assay for MCH1 receptors using [3H]AMR-MCH-1 in vitro and ex vivo

INTRODUCTION
Melanin-concentrating hormone (MCH) is a nineteen amino acid cyclic neuropeptide derived from pre-pro MCH (Nahon, 1994). To date, two human MCH receptors have now been identified, MCH1 (SLC-1) and MCH2 (Chambers et al., 1999; Saito et al., 1999; Hill et al., 2001). There is a considerable body of evidence indicating a role for MCH in the control of food intake and energy balance: iv administration of MCH to rats increases food intake (Qu et al., 1996; Rossi et al., 1999); MCH mRNA is overexpressed in ob/ob mice and fasted mice (Qu et al., 1996); MCH overexpressing mice are hyperphagic, mildly obese, hyperglycaemic and insulin resistant (Ludwig et al., 2001); MCH knockout mice are leaner than wild-type mice due to hypophagia and increased metabolic rate (Shimada et al., 1998). The MCH receptor appears to mediate the orexigenic effects of MCH (Chambers et al., 1999). Numerous MCH receptor antagonist for the potential treatment of obesity have appeared in recent years with at least two compounds entering clinical development (Menendez-Ardinino and Wos, 2007). Previously iodinated ligands have been used to label MCH1 receptors. Here we describe the development and validation of an in vitro radioligand receptor binding assay for MCH1 receptors using [3H]AMR-MCH-1 and human cloned MCH1 receptors. Using a modification of this assay the ex vivo occupancy of striatal MCH1 receptors by SCHA has been determined in dietary-induced obese C57BL/6j mice using the technique of autoradiography.

METHODS

RECEPTOR BINDING METHODOLOGY
Membrane preparation: MCH1 membranes (Batch 1138, Euroscreen, UK) were resuspended in ice-cold 50 mM Tris-HCl, pH 7.4 and placed on ice for 5 minutes prior to use in the binding assay. Saturation binding studies: Membranes (400µl; equivalent to 8.92µg tissue/lube) were incubated with 50µl of [3H]AMR-MCH-1 (6 concentrations 0.25 – 16 nM) and 50µl of incubation buffer (total binding) or 50 µl of SCHA (50 µM; non-specific binding) at 25ºC for 60 minutes. Recovery of membrane-bound radioactivity was recovered by rapid filtration under vacuum through Skatron 1173 filters pre-soaked in 0.5% polyethylenimine, using a Skatron cell harvester (setting 9,9,0). Filters were then washed with ice-cold 50mM Tris-HCl, pH 7.4. PRELIMINARY EXPERIMENTS
Effect of tissue concentration: Binding of [3H]AMR-MCH-1 (1–10 µl, Kd and 10 x Kd) to MCH1 receptors was evaluated using a range of tissue concentrations (2.23, 4.46, 8.92 and 13.37 µg/tube). Effect of incubation time: The binding of [3H]AMR-MCH-1 (1–10 µl, Kd and 10 x Kd) to MCH1 receptors was evaluated over different time periods (1, 2, 5, 10, 15, 20, 30, 45, 60, 75, 90, and 120 minutes). Data analysis: Equilibrium dissociation constants (Kd) and the maximal number of binding sites (Bmax) were determined by non-linear regression analysis. EX VIVO
Mice were given vehicle (po) or SCHA (10 or 30 mg/kg po) and terminated 6 or 24 hours later. Brains were removed. Coronal sections containing the caudate putamen were cut. Slides were incubated with [3H]AMR-MCH-1 (1.4 nM) and 50 mM Tris-HCl buffer, pH 7.4 (total binding) or SCHA (50µM; non-specific binding) at room temperature for 60 minutes. Binding was terminated by aspiration and sections washed in ice-cold buffer (4 x 15 mins). Radioactivity bound to the section was determined using a Beta-imager.

RESULTS
Binding of [3H]AMR-MCH-1 (0.15, 1.75 and 9.2 nM) increased linearly with protein over the range 4.46 to 13.37 µg protein/lube (Figure 1). A protein concentration of 8.92 µg protein/lube was chosen. Under these conditions specific binding was good (96±1% 0.15nM, 95±1% 1.75nM (Table 1) and 89±3% 9.2nM, respectively; mean ± SEM, n=4) and the total radioactivity bound as a percentage of the radioactivity added (84% ± 2, 95% ± 2, 94% ± 3%) was not significantly different. Therefore an incubation time of 60 minutes was chosen. Full saturation binding analysis revealed that binding approached saturation at the highest concentrations of radioligand. Binding was of high affinity and fitted well to a single site binding model (Kd = 1.42±0.08 nM and Bmax = 13.3±0.7 pmoles/mg protein; mean ± SEM, n=4). Binding of [3H]AMR-MCH-1 was high in the caudate putamen of brains from dietary-induced obese C57BL/6J mice in the vehicle-treated group, with low levels of non-specific binding as defined by SCHA (Figure 3A). SCHA (10 and 30 mg/kg po) occupied 95% and 102% at 6 hours and 46% and 74% at 24 hours post-dose, respectively of striatal MCH1 receptors when compared to controls (all p<0.001; n=5 mice per group; Figure 3B).

SUMMARY
These data indicate that [3H]AMR-MCH-1 is a high affinity, selective ligand (Sargent et al., this meeting) which can be used to label recombinant and native MCH1 receptors. The ex vivo binding data is in good agreement with Kovalski et al., 2006.

REFERENCES