Original article

MOLECULAR METABOLISM

Distribution and characterisation of Glucagon-like peptide-1 receptor expressing cells in the mouse brain

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ABSTRACT

Objective: Although Glucagon-like peptide 1 is a key regulator of energy metabolism and food intake, the precise location of GLP-1 receptors and the physiological relevance of certain populations is debatable. This study investigated the novel GLP-1R-Cre mouse as a functional tool to address this question.

Methods: Mice expressing Cre-recombinase under the *Glp1r* promoter were crossed with either a ROSA26 eYFP or tdRFP reporter strain to identify GLP-1R expressing cells. Patch-clamp recordings were performed on tdRFP-positive neurons in acute coronal brain slices from adult mice and selective targeting of GLP-1R cells *in vivo* was achieved using viral gene delivery.

Results: Large numbers of eYFP or tdRFP immunoreactive cells were found in the circumventricular organs, amygdala, hypothalamic nuclei and the ventrolateral medulla. Smaller numbers were observed in the nucleus of the solitary tract and the thalamic paraventricular nucleus. However, tdRFP positive neurons were also found in areas without preproglucagon-neuronal projections like hippocampus and cortex. GLP-1R cells were not immunoreactive for GFAP or parvalbumin although some were catecholaminergic. GLP-1R expression was confirmed in whole-cell recordings from BNST, hippocampus and PVN, where 100 nM GLP-1 elicited a reversible inward current or depolarisation. Additionally, a unilateral stereotaxic injection of a cre-dependent AAV into the PVN demonstrated that tdRFP-positive cells express cre-recombinase facilitating virally-mediated eYFP expression.

Conclusions: This study is a comprehensive description and phenotypic analysis of GLP-1R expression in the mouse CNS. We demonstrate the power of combining the GLP-1R-CRE mouse with a virus to generate a selective molecular handle enabling future *in vivo* investigation as to their physiological importance.

Keywords Glucagon-like peptide-1 receptor; Electrophysiology; Channelrhodopsin; Preproglucagon; GLP-1; PPG

1. INTRODUCTION

Glucagon-like peptide 1 (GLP-1) is traditionally recognised as a peripheral incretin hormone. Released postprandially from intestinal L-cells, it binds to GLP-1 receptors (GLP-1R) on pancreatic β-cells to increase insulin secretion. Additionally, GLP-1 acts as a neuropeptide and is produced by preproglucagon (PPG) neurons located in the lower brainstem, primarily in the caudal nucleus tractus solitarii (NTS) and the intermediate reticular nucleus [1,2].

Numerous rodent studies have addressed the question of GLP-1's action within the brain (for review see: [3,4]). Most studies have examined the effects of cerebroventricular injection of GLP-1 or its stable analogue exendin-4 (Ex-4) on food intake [5], but other reported effects include changes in heart rate and blood pressure, as well as effects on learning, memory and thermogenesis [6–9]. Furthermore, central GLP-1 appears to be involved in mediating the rewarding effect of food. Activation of GLP-1R within the ventral tegmental area (VTA) and nucleus accumbens (NAc) results in a significant decrease in the consumption of high calorie food and reduced body weight gain over 24 h [10].

Although the exact mechanism by which GLP-1 affects the brain is unclear, it has been demonstrated that within the VTA and NAc, GLP-1 appears to bind to presynaptic GLP-1R on glutamatergic terminals facilitating glutamate release, without triggering direct postsynaptic effects [11,12].

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Abbreviations: AP, area postrema; BNST, bed nucleus stria terminalis; DMH, dorsomedial nucleus of the hypothalamus; DMV, dorsal motor nucleus of the vagus; Ex-4, Exendin-4; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; GLP-1, Glucagon-like peptide-1; GLP-1R, Glucagon-like peptide-1 receptor; NAc, nucleus accumbens; NTS, nucleus of the solitary tract; PARV, parvalbumin; PPG, preproglucagon; PVN, paraventricular nucleus of the hypothalamus; TH, tyrosine hydroxylase; VTA, ventral tegmental area; YFP, yellow fluorescent protein

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It has previously been shown that GLP-1 producing neurons in the NTS send ascending axons to forebrain and brainstem regions associated with metabolic and autonomic control in the mouse, including the arcuate nucleus (ARC), hypothalamic paraventricular nucleus (PVN), rostral ventrolateral medulla and dorsomedial hypothalamus (DMH) [1,13]. These projections correlate well with the pattern of GLP-1R expression throughout the brain of the rat [14].

Given the potent effects of central GLP-1 on food intake, it is important to fully elucidate the exact expression profile of GLP-1R throughout the brain. The distribution of GLP-1R mRNA has previously been assessed in the rat brain by *in situ* hybridisation [14]; however, little is known about the distribution in mouse. This gap in knowledge is notable, especially given the increased usage of transgenic mouse models in metabolic physiology.

Here we detail the distribution of GLP-1R expressing cells throughout the mouse brain using a novel transgenic model, in which cre-recombinase is expressed under the control of the *Glp1r* gene. We show that GLP-1R expression correlates well with that observed in the rat [14], non-human primates [15] and with the projection pattern of mouse PPG neurons [1,13]. We also demonstrate that GLP-1R expressing cells do not exhibit immunoreactivity for glial fibrillary acid protein (GFAP) or parvalbumin (PARV), but do show tyrosine hydroxylase (TH) immunoreactivity in some areas. Furthermore, we establish that this mouse model is amenable to manipulation of specific subsets of GLP-1R expressing cells with cre-dependent viral gene transfer. Finally, we assess the feasibility of using this mouse model for *in vitro* analysis using brain slice patch-clamp recordings by recording electrical responses to exogenous GLP-1 from GLP-1R expressing cells in several brain regions.

2. MATERIAL AND METHODS

2.1. Animals

All animal experimentation was carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986, and had the required ethical approval. The entire study was performed on GLP-1R-CRE mice that express cre-recombinase under the *Glp1r* promoter. Generation of these mice has been described in detail before [16]. These mice were crossed with ROSA26-tdRFP or ROSA26-eYFP reporter strains [17] to enable fluorescent detection of cells expressing GLP-1R by cytosolic tdRFP or eYFP expression, respectively. A total of 3 GLP-1R-CRE-eYFP and 8 GLP-1R-CRE-tdRFP mice of both sexes were used for the immunofluorescence study, whereas the electrophysiological study was performed entirely with the GLP-1R-CRE-tdRFP strain. Mice were kept on a 12 h light — 12 h dark cycle, had access to food and water *ad libitum*, and were 8—16 weeks old (weighing between 25 and 35 g) when used for experiments. Females were consistently lighter than males.

2.2. Immunofluorescence

Mice were anaesthetised with urethane (1.5 g/kg; i.p.) and perfuse-fixed transcardially with heparinised 0.1M phosphate buffer (PB) followed by 4% paraformaldehyde in 0.1M PB, pH 7.4. Brains were removed from skulls, post-fixed overnight in the same fixative before being cryoprotected in 30% sucrose in 0.1M PB. Brains were sectioned coronally at a thickness of 30 μm (1:5 series) from the olfactory bulbs to the spinomedullary junction.

2.2.1. Immunohistochemical identification of eYFP or tdRFP

Tissues were blocked with 0.1% Triton X-100 and 10% normal goat serum diluted in 0.1M PB for 1 h at room temperature. GLP-1R-expressing cells were detected with antibodies raised against either green fluorescent protein (GFP; Catalogue #AB13970, lot #623923; Abcam, Cambridge MA, USA) or dsRED (Clontech #632496). These were added to the blocking solution in a 1:1000 dilution and incubated overnight at 4 °C. Subsequently, sections were washed 3 times for 5 min in 0.1M PB at room temperature, followed by incubation with a fluorescently labelled secondary antibody (1:500 dilution) in blocking solution for 2 h. Either an anti-chicken Alexa Fluor 488 (Catalogue# **A-11039**, Invitrogen) or an anti-rabbit Cy3 (Catalogue# C2306, Sigma Aldrich, St Louis MO, USA) was used. Sections were washed again as previously described, then mounted onto polylysine-coated slides (Catalogue# 631-1349, VWR), air dried and coverslipped with Vectashield mounting medium (Catalogue# H-1200, Vector Labs).

2.2.2. Double immunofluorescence

Staining was performed as described above with the exception of using sheep serum rather than goat for TH staining. Sections were then incubated with anti-GFP and anti-TH (1:1000 dilution, catalogue# sc-14007, Santa Cruz) or anti-GFP and anti-GFAP conjugated to Cy3 (catalogue# C9205, Sigma Aldrich, St Louis MO, USA), or anti-dsRED and anti-parvalbumin (1:5000 dilution, catalogue# 235, Swant, Switzerland) overnight at 4 °C. Following primary antibody incubation sections were washed as previously described and incubated with the appropriate secondary antibody: for TH the anti-rabbit conjugated to Cy3, and for parvalbumin the Alexa Fluor 488 goat anti-mouse (catalogue# A10680, Invitrogen), respectively. Sections were then washed and mounted as described above.

2.2.3. Data collection and analysis

Stained sections were analysed on an upright epi-fluorescence microscope (Leica DMRB, Leica Microsystems, Milton Keynes, UK) equipped with a Retiga 3000 colour digital camera (QImaging, Surrey, Canada). Images were captured using QCapture software (QImaging) and subsequently exported to Corel Photo-Paint X3 (Corel Corporation, Ottawa, Canada) where brightness and contrast were adjusted. Microsoft Image Composite Editor (Microsoft Corporation, Redmond, USA) was used to produce montages from several frames.

2.3. Viral targeting

Adeno-associated virus (AAV) particles were produced as described by Murray et al. [18]. Briefly, to produce rAAV virions containing a 1:1 ratio of type 1 and type 2 capsid proteins human embryonic kidney (HEK) 293 cells were co-transfected with the AAV plasmid pAAV-EF1a-double floxed-hChR2(H134R)-EYFP-WPRE-HGHpA (Addgene plasmid # 20298) and AAV1 (pH21), AAV2 (pRV1) helper plasmids as well as the adenovirus helper plasmid pFΔ6 using calcium phosphate. 64 h post-transfection, cells were harvested, digested with sodium deoxycholate allowing AAVs to be purified from lysates using 1 ml HiTrap heparin columns (Sigma). Eluted virions were concentrated using Amicon Ultra-4 centrifugal filter devices (Millipore U FC810024). Adult GLP-1R-CRE—tdRFP mice were anaesthetised with ketamine (75 mg/kg; i.m.) and medetomidine (0.5 mg/kg; i.m.). Deep anaesthesia was confirmed by the absence of a pedal withdrawal reflex to a toe pinch. The head was fixed in a stereotaxic frame and the skull was exposed to allow a cranial window to be drilled at the correct location for a unilateral microinjection (150 nl; 50 nl/min) of a solution containing viral particles of EF1a-double floxed-hChR2(H134R)-EYFP AAV serotype 1/2 to target GLP-1R neurons. The coordinates used to target the PVN were 0.82 mm caudal and 0.1 mm lateral from Bregma and 4.75 mm ventral from the surface of the skull at Bregma. After removal of the injection needle the skin was sutured and anaesthesia reversed

