Acute activation of GLP-1-expressing neurons promotes glucose homeostasis and insulin sensitivity

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ABSTRACT

Objective: Glucagon-like peptides are co-released from enteroendocrine L cells in the gut and preproglucagon (PPG) neurons in the brainstem. PPG-derived GLP-1/2 are probably key neuroendocrine signals for the control of energy balance and glucose homeostasis. The objective of this study was to determine whether activation of PPG neurons per se modulates glucose homeostasis and insulin sensitivity in vivo.

Methods: We generated glucagon (Gcg) promoter-driven Cre transgenic mice and injected excitatory hM3Dq-mCherry AAV into their brainstem NTS. We characterized the metabolic impact of PPG neuron activation on glucose homeostasis and insulin sensitivity using stable isotopic tracers coupled with hyperinsulinemic euglycemic clamp.

Results: We showed that after ip injection of clozapine N-oxide, Gcg-Cre lean mice transduced with hM3Dq in the brainstem NTS downregulated basal endogenous glucose production and enhanced glucose tolerance following ip glucose tolerance test. Moreover, acute activation of PPG neurons in the NTS enhanced whole-body insulin sensitivity as indicated by increased glucose infusion rate as well as augmented insulin-suppression of endogenous glucose production and gluconeogenesis. In contrast, insulin-stimulation of glucose disposal was not altered significantly.

Conclusions: We conclude that acute activation of PPG neurons in the brainstem reduces basal glucose production, enhances intraperitoneal glucose tolerance, and augments hepatic insulin sensitivity, suggesting an important physiological role of PPG neurons-mediated circuitry in promoting glycemic control and insulin sensitivity.

Keywords: Preproglucagon neurons; Glucagon-like peptides; Glucagon-Cre mice; Insulin sensitivity; Endogenous glucose production; Gluconeogenesis

1. INTRODUCTION

In response to food intake, glucagon-like peptides (GLP-1/2) are co-released from enteroendocrine L cells in the gut and preproglucagon (PPG) neurons in the nucleus of the solitary tract (NTS) of the brainstem, which together constitute the key nutritional signals for the control of energy balance and glucose homeostasis. Notably, GLP-1 receptor (GLP-1R) and GLP-2 receptor (GLP-2R) agonists are approved by the FDA for the treatment of type 2 diabetes and short bowel syndrome, respectively. PPG neurons widely project to central autonomic regions where Gip1r/2r are expressed [1–3]. PPG neurons are depolarized by leptin and may play a role in energy homeostasis and peripheral metabolism [4–7]. Intracerebroventricular (icv) infusion of exogenous GLP-1 or GLP-2 enhances glucose tolerance and insulin sensitivity [8–10]. However, it is unknown if PPG neurons play a physiological role in peripheral glucose metabolism and insulin sensitivity, though the physiological significance of endocrine GLP-1/2 is highlighted in maintaining glucose homeostasis. Increased gluconeogenesis is a primary feature of fasting hyperglycemia and type 2 diabetes (up to 40% of diabetic patients) [11]. Thus,
it is important to quantify in vivo gluconeogenesis [12]. Pyruvate tolerance challenge has been used as an indirect measurement for gluconeogenesis in mouse models. Except for glucose concentration, it does not actually quantify any metabolic flux of de novo glucose production. Stable isotopic tracers enable in vivo quantification of fractional gluconeogenesis in humans (e.g., by measuring the incorporation of deuterium from the body water into newly formed glucose) [12]. In order to define the physiological impact of PPG neurons that express the glucagon (Gcg) gene (also called Gcg neurons) in vivo, we wanted to quantify glucose kinetics and insulin sensitivity using dual stable isotopic tracers in conjunction with hyperinsulinemic euglycemic clamp.

The designer receptors exclusively activated by designer drugs (DREADD) approach has been developed for remote control of targeted neurons in the mouse brain for mapping feeding circuitry [13-19], and has been used to dissect the acute, neural control of peripheral metabolism [20-22]. Increasing evidence indicates that acute activation of distinct populations of neurons in the brain influences feeding behavior, food intake and body weight. To elucidate if PPG neurons regulate peripheral glucose metabolism, we wanted to create a genetic mouse model to enable their remote activation in a Gcg-dependent manner. Our objective was to define if acute activation of PPG neurons enhances peripheral glycemic control and insulin sensitivity in lean mice.

In the present study, we established a pharmacogenetics mouse model for the remote control of activation of PPG neurons in vivo. We first generated glucagon (Gcg) promoter-driven Cre transgenic mice and used them to create a mouse model for remote control of activation of PPG neurons using the DREADD approach. Moreover, we characterized the physiological significance of acute activation of PPG neurons on glucose metabolism and insulin sensitivity using stable isotopic tracers (6,6-2H2-D-glucose and 2H2O). We showed in Gcg-Cre lean mice infected with excitatory hM3Dq virus in the brainstem NTS that acute activation of Gcg neurons enhances glucose tolerance, suppresses basal endogenous glucose production, and augments hepatic insulin sensitivity. We conclude that acute activation of PPG neurons in the brainstem NTS promotes glucose homeostasis and insulin sensitivity, suggesting a physiological role of PPG neurons-mediated circuitry in glycemic control.

2. MATERIAL AND METHODS

2.1. Animals

The protocols of this study were approved by the Animal Care and Use Committee of Baylor College of Medicine and carried out in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Bethesda, MD). Glucagon (Gcg)-Cre mice were generated using the Gcg-Cre promoter-driven Cre transgenic embryos (ID 358-UNC donated by Pedro L. Herrera). Mice were maintained under a 12h:12h light-dark cycle with room temperature (~22 °C) and humidity (~50%) conditions and provided ad libitum access to water and a standard chow. To determine the metabolic impact of acute activation of targeted neurons, excitatory AAV-hM3Dq-mCherry viruses (serotype 8) were injected into the brainstem NTS of Gcg-transgenic mice at the age of ~8 wk. Two weeks later, the mice were fitted with the jugular cannulation for primed-continuous infusion of stable isotopic tracers. After one-week recovery, glucose tolerance test and hyperinsulinemic euglycemic clamp in conjunction with stable isotopic tracers were employed to quantify glucose homeostasis and insulin sensitivity in conscious mice with remote activation of targeted neurons. After mice were euthanized under isoflurane anesthesia, blood samples were dissected for immunohistochemistry and electrophysiology. Experimental Procedures in detail are provided in the Supplementary Material.

2.2. Immunohistochemistry

The whole brain was harvested 0.5 h after ip injection of clozapine-N-oxide (CNO, 0.3 mg/kg), fixed and cut at 25 μm for coronal sections. Brain slices were immunostained for c-fos and mapped for PPG neurons-innervated central autonomic regions [8].

2.3. Glucose tolerance test

Three weeks after viral injection, mice were fasted with free access to water. After 6-h fast, basal blood glucose concentrations were measured. 30 min after ip injection of CNO (0.3 mg/kg) or vehicle (PBS), mice were then challenged (at 1.5 g/kg BW, i.p.), and blood glucose concentrations were measured at the time points of 15, 30, 45, 60, 90, and 120 min.

2.4. Stable isotopic tracers in conjunction with hyperinsulinemic euglycemic clamp

Glucose metabolic fluxes were quantified with stable isotopic tracers in conjunction with hyperinsulinemic euglycemic clamp. Glucose metabolic fluxes and insulin sensitivity were assessed in mice after an overnight fast under ip injection of CNO (0.3 mg/kg) or saline (n = 8–10/group). A dual stable isotopic tracer method (6,6-2H2-D-glucose and 6,6-2H2-2H2O-glucose) coupled with hyperinsulinemic euglycemic clamp was employed to quantify glucose metabolism (including gluconeogenesis) and insulin sensitivity at a steady-state in conscious mice. In brief, mice were primed-continuously infused with 6,6-2H2-D-glucose via a jugular catheter for 3 h during basal period; and then for 3 h during insulin clamp (see Figure 4), whereas 6,6-2H2-D-glucose plus 2H2O-glucose were infused to maintain blood glucose level at ~100 mg/dL (see Fig. S2). Blood samples (5 μL each) were collected at 0, 3, and 6 h post infusion for analyzing isotopic enrichment of 2H2O with IR-MS; and spotted at 2:50, 2:25, and 3:00 h for the baseline; and at 5:50, 5:55, and 6:00 h for the clamp to analyze isotopic enrichment of 6,6-2H2-D-glucose with GC–MS.

2.5. Calculations for glucose kinetics

\[ \text{GIR} = \frac{\text{MPE(glucM2)blood}}{\text{MPE(2H2O)blood}}; \]
\[ \text{GEP} = \frac{\text{Ra} \times \text{MPE(glucM2)blood}}{\text{MPE(2H2O)blood}}; \]
\[ \text{GNG} = \frac{\text{MEP}(2H2O)blood}{\text{MEP(glucM1)blood}}; \]

Where MPE(glucM2)blood is 2H2-glucose enrichment in blood (mole % excess, MPE); MPE(2H2O)blood is 2H2O enrichment in blood; MPE(glucM1)blood is 2H-glucose enrichment in blood; MPE(glucM1) is 2H-glucose enrichment in blood; MPE(2H2O) is 2H2O enrichment in blood. Glucose kinetics at the steady state is calculated for GIR (glucose infusion rate), Ra (rate of glucose disappearance), GEP (endogenous glucose production), and GNG (gluconeogenesis) [8,23,24].

2.6. Electrophysiological recordings

Gcg-Cre mice injected with excitatory AAV8-hM3Dq-mCherry viruses were used to validate acute activation of Gcg neurons upon CNO application. Membrane potential and firing rate of Gcg-mCherry+ neurons in the brainstem NTS were measured by the whole-cell current patch clamp [8].
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