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 neuronsNTS 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 incor-
poration 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, neutral control of periphery
metabolism [20–22]. Increasing evidence indicates that acute activ-
ation 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 neu-
rons 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
transgenic mouse model (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 pro-
vided ad libitum access to water and a standard chow. To determine
the metabolic impact of acute activation of targeted neurons, excit-
tory AAV-hM3Dq-mCherry viruses (serotype 8) were injected in to the
brainstem NTS of Gcg-transgenic mice at the age of 6–8 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, brain samples were
dissected for immunohistochemistry and electrophysiology. Exper-
imental 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
-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
sugar 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 meta-
bolic 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) 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
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.

2.5. Calculations for glucose kinetics

\[
\begin{align*}
(1) \ R_d &= R_a = GIR \cdot MPE(\text{glucM2})_{infusate}/MPE(\text{glucM2})_{blood}; \\
(2) \ EGP &= R_a - GIR; \\
(3) \ Fractional\ GNG &= 100 \cdot MPE(\text{glucM1})_{blood}/MPE(2H2O)_{blood}; \\
(4) \ GNG &= EGP/Fractional\ GNG; \\
\end{align*}
\]

Where MPE(\text{glucM2})_{infusate} is 2H2-glucose enrichment in infusate (in
mole % excess, MPE); MPE(\text{glucM2})_{blood} is 2H2-glucose enrichment in
blood; MPE(\text{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), Rf (rate of
glucose disappearance), Ra (rate of glucose appearance), EGP (endogenous glucose production), and GNG (gluc
oneogenesis) [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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