Do neurons involved in the regulation of body weight directly affect breathing?

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Introduction

Obstructive sleep apnea (OSA) occurs in 40-70% of the obese individuals and is the most common form of sleep disordered breathing

Obesity leads to neurological changes in the periaqueductal grey (PAG) and the arcuate nucleus (ARC) - both essential brain regions that modulate breathing.

Objective

The goal of the project is to determine how a subset of neurons which principally regulate body weight (the ARC_POMC) affects breathing.

Methods

Animals. 20-40 weeks old, POMC-Cre (strain number: 005965) and diet-induced obese (DIO) were ordered from Jackson Laboratory (Bar Harbor, ME) and placed on a standard diet (21% Fat, Picolab 5058) and obesity-inducing diet (60% Fat, Teklad, TD.82414), respectively. Mice were housed in 12L:12D light conditions, with lights on at 8am and lights off at 8pm.

Plethysmography. Baseline ventilation and the hypercapnic ventilatory response (HCVR) was measured in a freely moving, awake, conscious mouse using a whole-body plethysmograph (Emka Technologies, Falls Church, VA). After 30 min acclimation, baseline ventilation is measured while the mice are exposed to room air (e.g. 21% O2, 79% N2, 0% CO2). To determine HCVR, the CO2 concentration inside the chamber is elevated for periods of 4 minutes and separated by 5-minute recovery periods in room air. Mice are exposed to increments of 3%, 5%, and 8% CO2 while real-time ventilatory responses are recorded. As CO2 is increased, N2 is decreased to allow O2 levels to be stabilized at 21%. HCVR is calculated as the slope of the line (min mol% CO2).

Plethysmography of Hypercapnic Ventilatory Response

To determine the hypercapnic ventilatory response (HCVR), mice are exposed to progressively higher concentrations of CO2 while their breathing is measured. The slope of the response is calculated as the change in ventilation divided by the change in CO2 concentration.

Stereotaxic Surgery for Designer Receptor Exclusively Activated by Designer Drug (DREADD). For the ARC, 100 nl of virus was injected into an anesthetized mouse at an 8° angle, anteroposterior (AP) -1.5, mediolateral (ML) ±0.8, and dorsoventral (DV) -5.9. Both inhibitory (AVVh15m.DIO.M4D(Gq)-mCherry) and excitatory (AVVh15m.DIO.M3D(Gq)-mCherry) DREADDs were ordered from Addgene (Waterston, MA). Four weeks after surgeries, mice were chosen at random to receive either clozapine-n-oxide (CNO) (i.e. the designer drug) via an intraperitoneal (i.p.) injection to activate the DREADDs or the vehicle (normal saline), one hour before the plethysmography testing was performed.

Brain Removal/Imaging. Brains were removed from mice and placed in 4% paraformaldehyde overnight. Brains were then placed in a 20% sucrose solution and allowed to sink, for at least 2 days. Brains were rinsed in phosphate buffered solution (PBS) and placed in a small block of OCT embedding compound for cryossectioning. Brains were sliced into 40-μm sections on a cryostat (Leica) and placed into small wells containing phosphate buffered solution PBS, 1x. The slices were then mounted on glass slides and coverdipped with Fluorosave-G, with DAPI (Invitrogen). Slides were then imaged on a Nikon confocal microscope.

FIGURE 1. Exciting PAG_LepR in lean mice worsens chemosensitivity while inhibiting PAG_LepR in obese neurons improves chemosensitivity.

Conclusion

Based on our understanding, we have predicted three different outcomes that could potentially determine the role which the ARC plays in the hypothesized breathing circuit (figure 4). If our hypothesis is supported, inhibiting the metabolic ARC brain region may help restore healthy breathing to obese mice.

FIGURE 2. Targeted excitation of ARC_POMC neurons shows a trend to reduce hypercapnic ventilatory response (HCVR) in obese mice.

FIGURE 3. Hypothesized circuit of disordered breathing

Possible Outcomes of the Hypothesized Circuit: HCVR in Obese Mice

Our hypothesized prediction; removing the excitatory inputs to the PAG restores healthy breathing

Interpretation: The ARC does not excite the PAG, and/or another brain region primarily drives PAG excitation and disordered breathing

Conclusions

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