Role of raphe GABAergic neurons in leptin-regulated food intake: effect of L-allylglycine on GABA content

School of Medicine Columbia

Christa P. Joby, Maya R. Wright, Nicholas D. Maxwell, Alia T. Sadek, Donzelle L. Benton, Lawrence P. Reagan, Claudia A. Grillo

College of Charleston Honors College

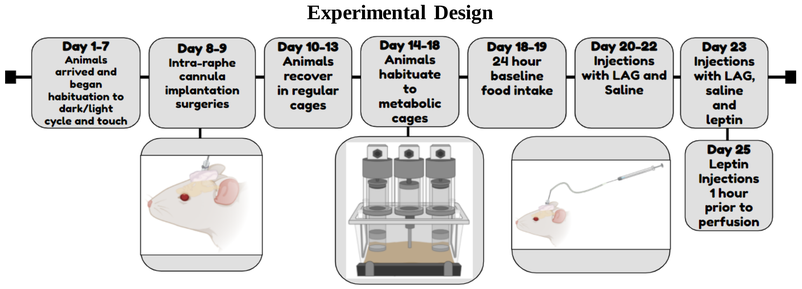
Pharmacology, Physiology & Neuroscience Department, University of South Carolina School of Medicine, Columbia, SC 29209, USA

Introduction

Feeding behavior is a major component of survival; however, when it is not well regulated, negative consequences can occur. It is vital to recognize that obesity, a condition of being overweight due to excessive accumulation of fat, and anorexia, a condition in which not enough nutrients are consumed to maintain normal body mass, are impacted by other factors besides self-control and lifestyle habits. Contributing factors to obesity include but are not limited to genetics, overeating, disease, pharmacological drugs, and sedentariness. Many physiological factors influence eating behavior, notably leptin. Leptin is a hormone secreted by adipose tissue in the body, and primarily works in the brain to reduce food intake. In obese individuals, leptin levels increase, and over time these individuals develop resistance to leptin. Aside from affecting appetite, leptin can regulate energy spending. Gamma-aminobutyric acid (GABA) is a neurotransmitter that functions to inhibit specific brain signals that can have a calming effect on the body. The enzyme glutamic acid decarboxylase (GAD) transforms glutamic acid into GABA, which then binds to GABA receptors, opening ligand-gated chloride channels, letting chloride ions enter the neuron. This action makes it less likely for the brain to respond to the stimuli. L-Allylglycine is an amino acid derivative that inhibits GAD. The focus of this experiment is to evaluate the importance of GABA in leptin's ability to reduce food intake in the dorsal raphe nucleus by reducing the amount of available GABA in the DRN before leptin administration.

Methods

Experimental Design



All animals used in this study were cared for following the protocols and procedures approved by the University of South Carolina Animal Care and Use Committee.

Habituation and Baseline: 18 Adult, male Sprague-Dawley rats were habituated individually and given access to food and water. Prior to noon each day, animals were weighed and handled.

Day 8-9: Intra-raphe cannula implantation surgeries were performed.

Day 10-18: Rats recovered from surgery in the regular cages. Rats were then relocated to metabolic cages to habituate to the new environment, hoppers, and water bottles.

Day 18-19: Food intake data of the rats in a 24-hour time frame was automatically recorded through the metabolic cages to establish a baseline food intake.

Day 20-23: L-allylglycine (LAG) was injected once per day for 4 days. 20 µg of LAG was given to half of the rats or vehicle was given to the other half. In addition to LAG injections on day 23, 5µg of leptin was administered prior to the dark cycle.

Euthanasia (Day 25): One hour prior to sacrifice, animals were administered 5µg leptin or vehicle into the DRN. One hour later, animals were given an overdose of anesthetic isoflurane. Transcardial perfusion via the left ventricle of the heart was performed with 0.1M PBS followed by 4% paraformaldehyde/0.1 M PBS (PFA). Brains were collected, post fixed for 24 hours in 4%PFA and placed in 30% sucrose/phosphate buffer solution for several days at 4ºC.

Tissue Preparation: Brains were removed from 4ºC and frozen using 2-methylbutane and stored at -80ºC. Each brain was sectioned at 20 um using a cryostat and immediately mounted onto slides.

Immunohistochemistry (IHC): IHC was performed on tissue sections containing raphe nuclei. Hydrophobic barriers were drawn on mounted slides prior to starting TBST washes. Samples were then placed in 4% PFA wash for 10 min/3 washes of TBST for 5 min/0.3% Glycine in TBS for 10 min/3 washes for 5 min each in TBST. Sections were incubated in primary with antibody solution overnight at 4°C. The following day, after 3 washes with TBST for 5 minutes, sections were incubated in secondary antibody for 4 hours at RT. Dilute secondary antibody in TBS and 0.5% Triton X-100/wash 3X for 5 min each in TBST.

Imaging: Labeled mounted sections on slides were cover slipped and imaged using Leica SP8 confocal microscope.

Results

Figure 1

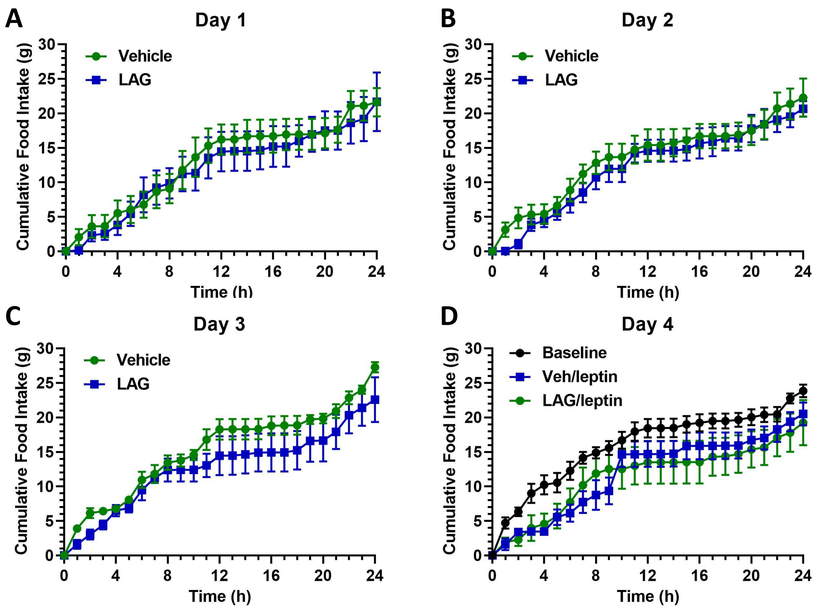


Figure 1. Food intake following administration of LAG, vehicle and leptin to the DRN. Food intake data collection begins (T=0) at 7pm (when dark cycle begins, and rats' activity increase) after LAG/vehicle injections were administered. (A) There is no difference in food intake between the vehicle treated and the LAG treated animals in day 1. (B) Day 2 shows some difference between food intake between LAG-treated rats and control rats around 1–2 hours after the dark cycle begins but normalize over time. (C) A larger gap between the LAG/vehicle starting at roughly 10 hours can be observed. This could indicate that LAG may affect feeding behavior after several days of administration. (D) The rats received leptin after LAG/vehicle, just before the dark cycle. Compared to baseline, both groups that received leptin saw decreased food intake. Those decreases were statistically significant for both group of rats at the time points between 2 and 7 hours, determined by a one-way ANOVA.

Figure 2

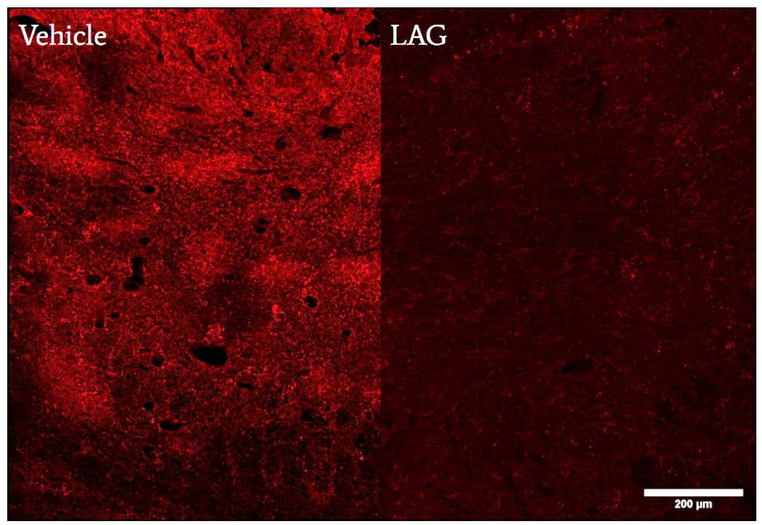
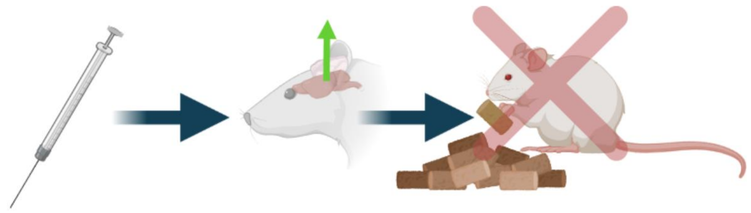


Figure 2: Representative images of immunohistochemical differences in GABA expression and distribution within the DRN. LAG treatment effectively decreases the levels of GABA in the DRN. Differences in intensity between treatment and vehicle animals can be observed. Scale bar = 200 µm.

Conclusions

- Leptin successfully decreased food intake when injected into the dorsal raphe nucleus for both vehicle and LAG treated rats in comparison to normal food intake.
- Behavioral food intake data has not indicated if GABA is involved in this leptin-regulated pathway.
- The content of GABA in the DRN is lowered as a result of LAG injections.
- GABAergic neurons that express leptin receptors are present in the DRN.
- Immunofluorescence images show less GABA present in LAG-treated rats compared to vehicle.



Future Directions

- Repeat experiment with four different treatment groups (vehicle-vehicle, vehicle-leptin, LAG-vehicle, LAG-leptin) and with larger n-sizes
- Conduct dose-response experiments to determine optimal concentrations of LAG.
- Conduct the experiment over a longer time frame.
- Use micropumps to inject LAG continuously, rather than once daily injections.

References

1. Münzberg H, Morrison CD. Structure, production and signaling of leptin. Metabolism. 2015 Jan;64(1):13-23. doi: 10.1016/j.metabol.2014.09.010. Epub 2014 Sep 28. PMID: 25305050; PMCID: PMC4267896.
2. Hristov, M., Landzhov, B., Nikolov, R. et al. Central, but not systemic, thermoregulatory effects of leptin are impaired in rats with obesity: interactions with GABA B agonist and antagonist. Amino Acids 51, 1055–1063 (2019). https://doi.org/10.1007/s00726-019-02746-x
3. Liu J, Yang X, Yu S, Zheng R. The Leptin Signaling. Adv Exp Med Biol. 2018;1090:123-144. doi: 10.1007/978-981-13-1286-1_7. PMID: 30390288.
4. Stanley BG, Urstadt KR, Charles JR, Kee T. Glutamate and GABA in lateral hypothalamic mechanisms controlling food intake. Physiol Behav. 2011 Jul 25;104(1):40-6. doi: 10.1016/j.physbeh.2011.04.046. Epub 2011 May 4. PMID: 21550353.
5. Woods SC, Seeley RJ. Hap1 and GABA: thinking about food intake. Cell Metab. 2006 Jun;3(6):388-90. doi: 10.1016/j.cmet.2006.05.007. PMID: 16753573.
6. Cruz-Martínez AM, Tejas-Juárez JG, Mancilla-Díaz JM, Florán-Garduño B, López-Alonso VE, Escartín-Pérez RE. CB1 receptors in the paraventricular nucleus of the hypothalamus modulate the release of 5-HT and GABA to stimulate food intake in rats. Eur Neuropsychopharmacol. 2018 Nov;28(11):1247-1259. doi: 10.1016/j.euroneuro.2018.08.002. Epub 2018 Sep 11. PMID: 30217553.
7. Abshire VM, Hankins KD, Roehr KE, DiMicco JA. Injection of L-allylglycine into the posterior hypothalamus in rats causes decreases in local GABA which correlate with increases in heart rate. Neuropharmacology. 1988 Nov;27(11):1171-7. doi: 10.1016/0028-3908(88)90013-5. PMID: 3205383.
8. National Center for Biotechnology Information. "PubChem Compound Summary for CID 167529, L-Allylglycine" *PubChem*,
9. Paine, T. A., O'Hara, A., Plaut, B., & Lowes, D. C. (2015). Effects of disrupting medial prefrontal cortex GABA transmission on decision-making in a rodent gambling task. Psychopharmacology, 232(10), 1755–1765. https://doi.org/10.1007/s00213-014-3816-7

Acknowledgments

We are thankful to be collaborating with the Department of Pharmacology, Physiology and Neuroscience at the University of South Carolina School of Medicine. This project was support by the National Science Foundation Grant Number IOS-1656626 (CAG).