The Physiological Effects and Neuronal Activity of Rats Treated with GLP-1 and Ex-4: A Novel Treatment for Obesity

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Introduction

Background:

- Obesity currently affects 42% of adults and 19% of children in the United States [1] United States PDA-approved glucagon-like peptide-1 receptors (GLP-1R) are successful for diabetes and obesity treatments. However, its efficacy is limited by its side effects, such as nausea, vomiting, and malaise.
- Exendin-4 (Ex-4) is an agonist of GLP-1R. Ex-4 has a much longer half-life than GLP-1, so it is more effective in reducing blood glucose levels in patients with type 2 diabetes. [2]
- The locus coeruleus (LC) of the brain is a site of action for GLP-1 signaling and a primary source of norepinephrine (NE) in the brain. Recent studies have demonstrated the role of the LC in modulating food intake. [1] Since LC has an established role in coordinating autonomic responses, the ability of LC Ex-4 to affect sympathetic and parasympathetic outflow is examined.

Objectives of the study:

- The autonomic/ physiological effects of GLP-1 and Ex-4 in rats
- c-Fos expression, which shows neuronal activity in the LC in rats

Hypothesis:

- Activation of GLP-1Rs in the LC suppresses food intake and body weight
- Abundance c-Fos expression in the LC with rats treated with Ex-4 but not GLP-1

Methods

- Injection: 22 rats that are implanted with an indwelling cannula aimed at the LC are injected with 100ng of GLP-1, 0.05 µg of Ex-4, and vehicle treatments
- Terminal: perfusion and extraction of 22 rat brains is carried out. Paraformaldehyde is used to fix the brain tissues
- Brain slicing: The locus coeruleus (LC) of 22 rat brains are sliced at 30 microns on a cryostat for imaging
- Immunohistochemistry: A technique using antibodies to detect antigens in the rat tissue samples. c-Fos and DBH are the antibodies. These proteins are markers of neuronal activity that are easily expressed after a wide range of stimuli.
- Brain imaging and quantification using KEYENCE BZ-X700

Image 1: Rats implanted with indwelling cannulae directly into the LC

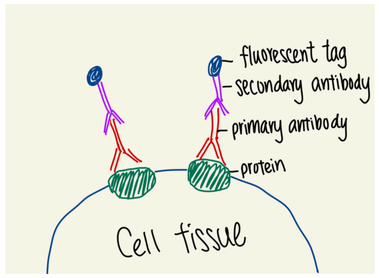


Image 2: Immunohistochemistry

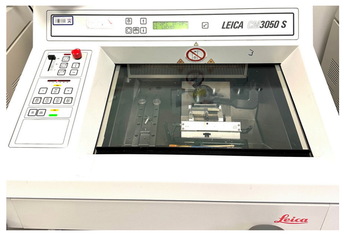


Image 3: Cryostat used to slice the rat brains

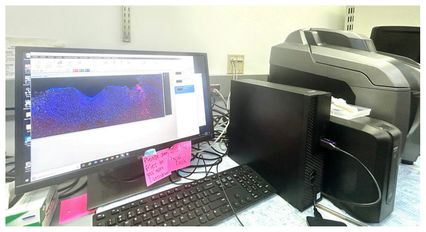
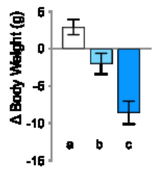


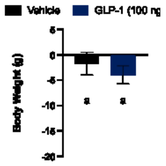
Image 4: KEYENCE used for brain imaging and quantification

Background Data

Graph 1: Effect of Ex-4 on body weight of rats

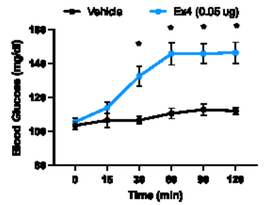


Graph 2: Effect of GLP-1 on body weight of rats

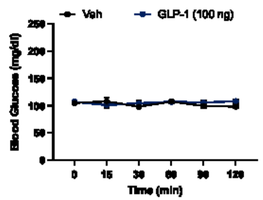


There is reduced body weight for Ex-4 but not GLP-1 for treated rats. The higher the concentration of Ex-4, the greater the weight loss.
In terms of food intake, the results showed a similar trend. The higher the concentration of Ex-4, the greater the reduction of food intake.
This supports the first hypothesis - activation of GLP-1Rs in the LC suppresses food intake and body weight.

Graph 3: Effect of Ex-4 on blood glucose

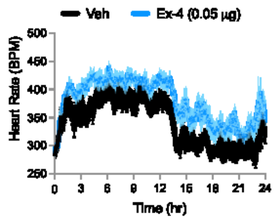


Graph 4: Effect of GLP-1 on blood glucose

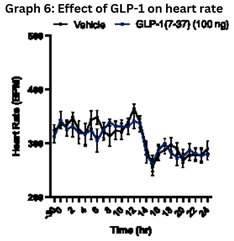


Ex-4 increases blood glucose levels. In contrast to Ex-4, GLP-1 does not trigger any later rise in blood glucose relative to control animals.

Graph 5: Effect of Ex-4 on heart rate

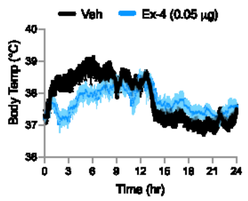


Graph 6: Effect of GLP-1 on heart rate

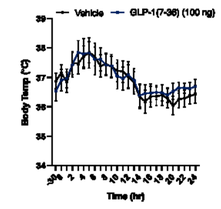


Rats injected with Ex-4 have higher heart rates than those with vehicle treatments. Ex-4 raises heart rates from 0-24 hours in rats. However, there is no visible increase in heart rate for rats treated with GLP-1

Graph 7: Effect of Ex-4 on body temperature



Graph 8: Effect of GLP-1 on body temperature



Rats treated with Ex-4 and GLP-1 have an increased body temperature from the 12th-24th hours.

Results: Neuronal Activity

Figure 1: Brain Image of rat with vehicle treatment - Overlay

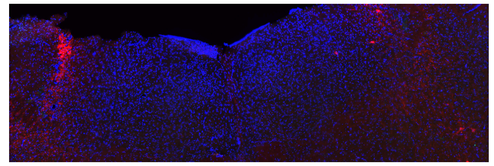


Figure 2: Brain Image of rat treated with GLP-1 - Overlay

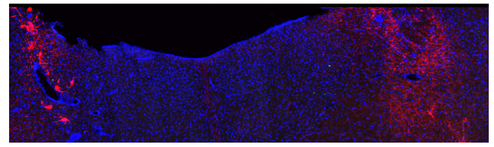
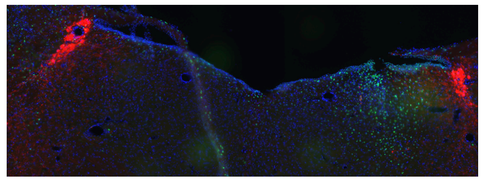


Figure 3: Brain Image of rat treated with Ex-4 - Overlay



Key:
Blue: Cell nuclei

Green: Cell activity

Red: Noradrenergic neurons

Conclusion

- Both GLP-1 and Ex-4 activate GLP-1 receptors. However, there are some differences:

1. For rats treated with Ex-4, there is reduced food intake and weight loss in rats, but not GLP-1
2. Ex-4 increases blood glucose levels but not GLP-1
3. Ex-4 increases heart rate but not GLP-1
4. Ex-4 and GLP-1 trigger a rise in body temperature from the 12th - 24th hours

--> For a long time, researchers have observed strange hyperglycemia in rats with Ex-4 and now there is a site of action - the LC

- Rats treated with Ex-4 have an abundance of c-Fos expression, and hence neuronal activity, while those treated with GLP-1 do not

--> Ex-4 is not degraded by the enzyme dipeptidyl peptidase 4 (DPP4), which breaks down peptides like GLP-1

--> consistent with studies showing how Ex-4 is associated with enhanced c-Fos reactivity [3]

- Future implications: developing treatments that can reduce side effects

Reference

[1] Fortin, S. M., Chen, J. C., Petticord, M. C., Ragozzino, F. J., Peters, J. H., & Hayes, M. R. (2023). The locus coeruleus contributes to the anorectic, nausea, and autonomic physiological effects of glucagon-like peptide-1. Science Advances, 9(38). Your paragraph text
[2] Pérez-Tilve, D., González-Matías, L., Aulinger, B. A., Alvarez-Crespo, M., Gil-Lozano, M., Alvarez, E., Andrade-Olivie, A. M., Tschöp, M. H., D'Alessio, D. A., & Mallo, F. (2010, May). Exendin-4 increases blood glucose levels acutely in rats by activation of the sympathetic nervous system. American journal of physiology. Endocrinology and metabolism.
[3] Kjaergaard, M., Salinas, C. B., Rehfeld, J. F., Secher, A., Raun, K., & Wulff, B. S. (2019). PYY(3-36) and exendin-4 reduce food intake and activate neuronal circuits in a synergistic manner in mice. Neuropeptides, 73, 89–95.

Acknowledgements

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