Effect of α-MSH and NPY on M1 Macrophages

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Introduction

The innate immune system is composed of a variety of cells that are the first line of defense to work against foreign substances. Immune privilege describes how the microenvironment of the eye regulates inflammation initiated from this system in order to preserve vision.

One of the mechanisms that inhibits inflammation is through immunosuppressive molecules, such as alpha-melanocyte stimulating hormone (α-MSH) and Neuropeptide-Y(NPY).

We used macrophages polarized to be pro-inflammatory M1 macrophages using lipopolysaccharide (LPS) and interferon-γ. Macrophages produce cytokines which are small molecules that signal responses to other immune cells. IL-6 and TNF-α are pro-inflammatory cytokines while IL-10 is an anti-inflammatory cytokine.

In this study, we examined whether NPY or α-MSH altered the production of specific cytokines including IL-6, TNF-α, and IL-10 to further understand if the suppression from these neuropeptides is by suppressing production of pro-inflammatory cytokines or by enhancing the production of anti-inflammatory cytokines to reduce inflammation.

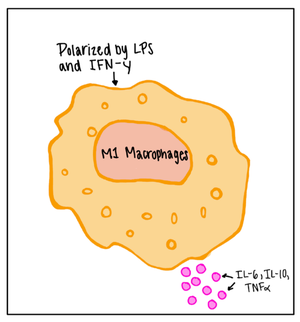


Figure 1: Macrophages are a type of white blood cell that are formed from monocytes and turn into macrophages when they enter tissue. The macrophages in this experiment were polarized through LPS and IFN-γ to become M1 (pro-inflammatory).

Methods

M1 Polarization:

The macrophage cell line, RAW cells, were added into 10 wells within a 24-well plate. The groups were then treated with LPS and IFN-γ to polarize into M1 macrophages.

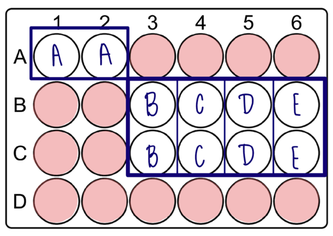


Figure 2: This is a pictorial representation of the 24-well plate the cells were polarized and treated in. The groups were treated with their corresponding neuropeptides, A (cells), B (untreated), C (+α-MSH+ NPY), D (+α-MSH), and E (+NPY).

Neuropeptide Treatment:

Following 48 hours of incubation at 37˚C and 5% CO_2, a Nitric Oxide (NO) assay was performed for culture supernatant for NO generation, a pro-inflammatory chemical to verify the polarization. Once confirmed, they were treated with their proper neuropeptides, either NPY and/or α-MSH, or neither (negative and positive controls).

ELISA: Following a 24-hour incubation period at 37˚C and 5% CO_2, the culture supernatant was collected and a sandwich ELISA was performed for either IL-6, IL-10, or TNF-α to measure the cytokine concentration. The concentration levels were then analyzed.

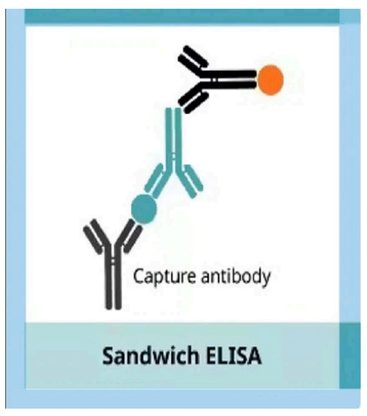


Figure 3. A sandwich ELISA consists of a capture antibody, the antigen, and a detection antibody. It is called a sandwich because your antigen is bound between the capture and detection antibodies.

Results

ELISA Images

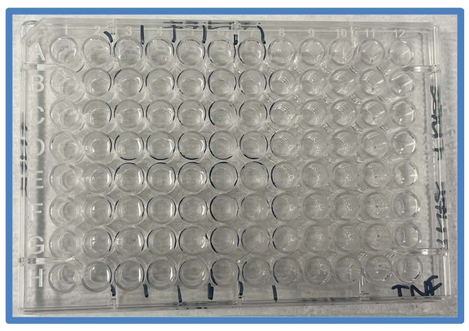


Figure 4. Immediately after adding the substrate

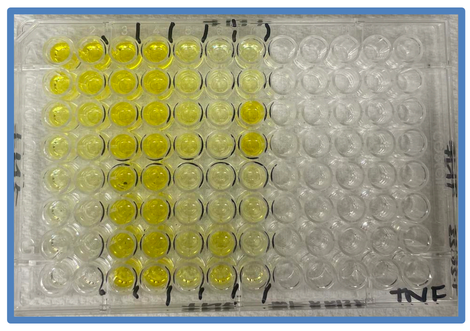


Figure 5. 20 minutes after adding the substrate

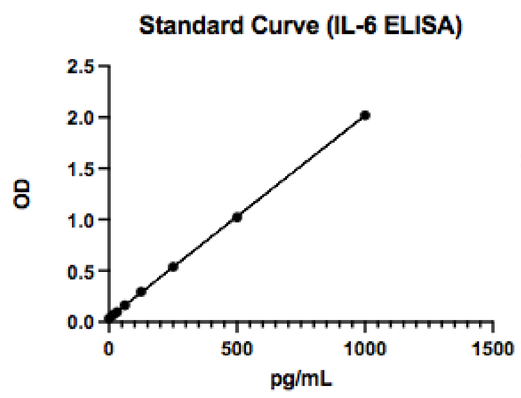
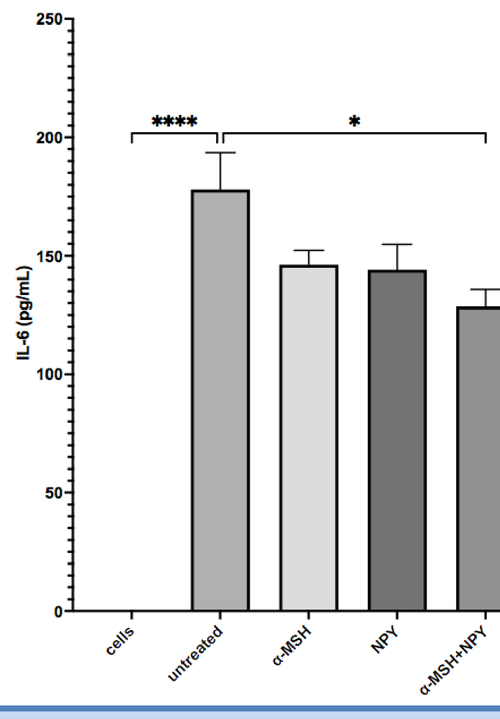


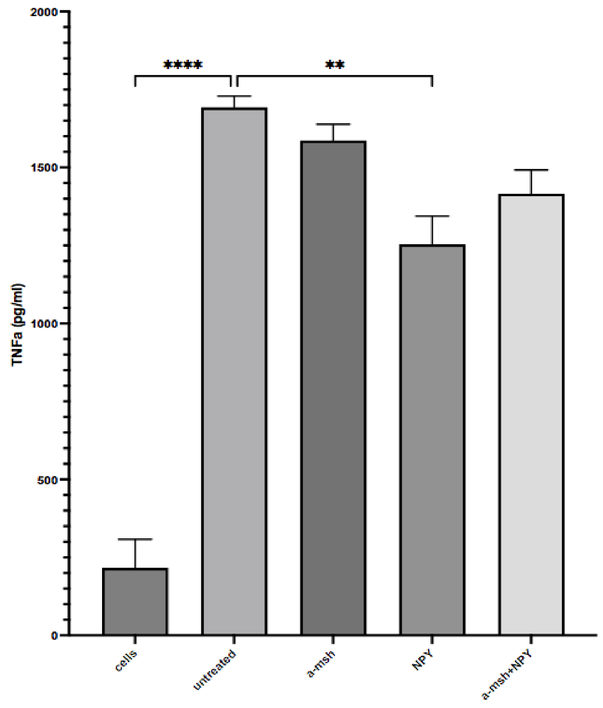
Figure 6. IL-6 ELISA Standard Curve graph

Figure 7. Effect of α-MSH and NPY on IL-6 levels



The pro-inflammatory cytokine IL-6 was significantly suppressed (n=4, $P \leq 0.05$) by the neuropeptide treatment of α-MSH and NPY compared to the untreated (positive control) group.

Figure 8. Effect of α-MSH and NPY on TNF-α levels



The pro-inflammatory cytokine TNF-α was significantly suppressed (n=4, $P \leq 0.05$) by the neuropeptide treatment of NPY compared to the untreated (positive control) group.

Summary

- The mechanism that the neuropeptides used to suppress the M1 pro-inflammatory activity was by decreasing the production of pro-inflammatory cytokines. This was shown by the significantly reduced production of the pro-inflammatory cytokine IL-6 in the α-MSH and NPY treated M1 macrophages compared to the untreated M1 macrophages.
- The usage of this mechanism was also supported by the data from the TNF-α ELISA as the production of the pro-inflammatory cytokine TNF-α was significantly reduced in the NPY treated M1 macrophages compared to the untreated M1 macrophages.
- There was no anti-inflammatory cytokine, IL-10, made by the M1 macrophages or the M1 macrophages treated with the neuropeptides α-MSH and/or NPY.
- This suggests the mechanism by which the neuropeptides suppress the inflammation is by inhibiting the production of pro-inflammatory cytokines from M1 macrophages.

Future Directions:

- Analyze concentration levels of other pro-inflammatory cytokines such as IL-1β after the same neuropeptide treatments
- Increase the concentrations of neuropeptide treatments to see if there is greater inhibition or if other groups become significant

Figure 9. Table with significant results

		Cells	Untreated	α-MSH+ NPY	α- MSH	NPY
Pro-inflammatory Cytokines	IL-6	ns	control	↓	ns	ns
	TNF-α	ns	control	ns	ns	↓
Anti-inflammatory Cytokine	IL-10	none	none	none	none	none

References

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- Zhou R, Caspi RR. Ocular immune privilege. F1000 Biol Rep. 2010 Jan 18;2:3. doi: 10.3410/B2-3. PMID: 20948803; PMCID: PMC2948372.

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