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Effect of Tyrosine on the Productivity of PC12 Cells by Measuring Extracellular ATP release

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Effects of Tyrosine on the Productivity of PC12 Cells by Measuring Extracellular ATP Release

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Abstract

Given the challenge to "reverse-engineer the brain", our group tried to address the problems of a lack of long term focus and attention span. Tyrosine was determined to aid in mental focus and brain productivity as it is a precursor to the neurotransmitter Dopamine. Given that Tyrosine is naturally occurring in the body, it is preferable to add to the body, whereas a substance like Adderall has negative effects, including increased heart rate that, over time, can cause cardiovascular damage. Drawing connections between in increase ATP and an increased ability to focus and perform under stress, extracellular ATP secretion of PC12 cells is measured using a luminometer. This research is novel and important as it attempts to find a way to increase the the human brain's ability to focus. Based on results, higher levels of tyrosine in the brain would lead to increase overall human cognitive function and productivity.

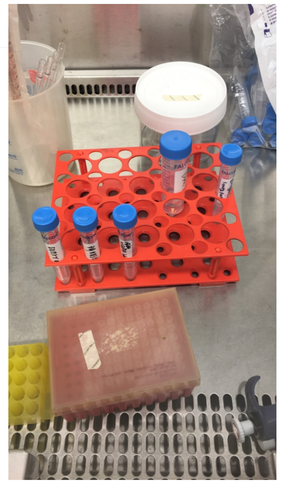
Introduction

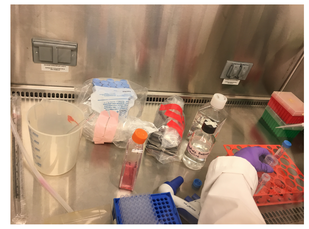
Tyrosine has been seen to increase catecholamine levels (i.e. dopamine, epinephrine, and norepinephrine) in the brain, which are produced by the body under stressful situations and tied to the ability to focus and perform (Hase et al. 2015). A study performed by the Department of Clinical Neuropsychology tested 21 military cadets in a combat training course and specifically under high stress conditions using tyrosine supplements. They reported that the cadets taking the tyrosine supplements had higher cognitive functions and performed better in the various tasks they were asked to do (Deijen et al. 1999). Furthermore, tyrosine is the precursor for L-Dopamine in the dopaminergic pathway. An increase in dopamine would theoretically lead to an overall increase in focus as well as mental ability and performance, including an increase in extracellular ATP production. ATP is a very important molecule within biochemical systems. It has been found to play a role in insulin secretion, neurotransmission, cell motility, and organ development. Cell culturing studies have started measuring the levels of ATP to assess the productivity of cells (Ostrom et. al 2000).

Figure 1 *shows the dopaminergic synthesis pathway by way of amino acid tyrosine.*

Methods

- We used cell culture to obtain quantitative data and to measure the molecular changes in cell productivity. Th PC12 cell line (adrenal gland tumor cells from a rat) were used as they have large vesicles to transport molecules like ATP across the cell membrane. The extracellular ATP secretion was measured in tyrosine-treated and untreated cell in a 96 well plate by a luminometer.
  - **Pretreatment:** tyrosine was dissolved in pH 10 water and cells were treated with this solution. For the control (untreated) cells, pH 10 water without tyrosine was used.
  - **Luminometer Luciferase Detection:**
    - Using a luminometer allowed us to see how extracellular ATP production increased and decreased when ATP reacted with the enzyme luciferase which was added to the wells in the 96 well plate. The interaction of ATP and luciferase creates light output that is then measured by the luminometer. This light output, measured in relative light units (RLU) was graphed and recorded for both tyrosine treated and untreated cells. Luminescence was measured with all wavelengths. Based on the numbers, we could generalize about ATP production in cells and how tyrosine was able to influence the results.
    - 4 µl of 1M KCl was used in order to open the cell membrane channels by depolarization, allowing for ATP to be released from the cell and for it to interact with the luciferase enzyme. Hepes in Dulbecco's phosphate-buffered saline (DPBS) is used as a buffer as the KCl creates a highly acidic environment and compromises the overall productivity of the cells by decreasing the cells' ability to produce ATP.
    - In both wells, KCl was added after a baseline is obtained. The baseline was measured until stable, in 30 second intervals. Once the KCl was added, the wells were measured for 10 minutes. Wells were measured one at a time, first being the control and second being the tyrosine treated.





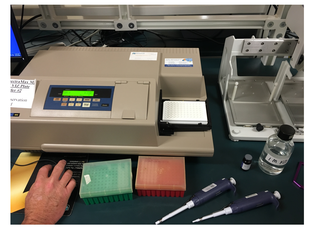


Figure 4: *Far left photo: different 15 mL falcon tubes with the various treatments. Photo on the top right: group member working in the fume hood. Photo on the bottom right: Use of the luminometer to read the 96 well plate.*

Results

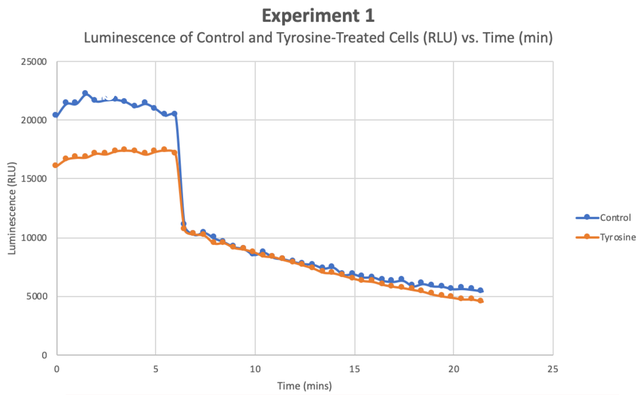


Figure 2 *shows the significant RLU drop after KCl was added due to the overwhelmingly acidic cell environment which overpowered the ATP light signal*

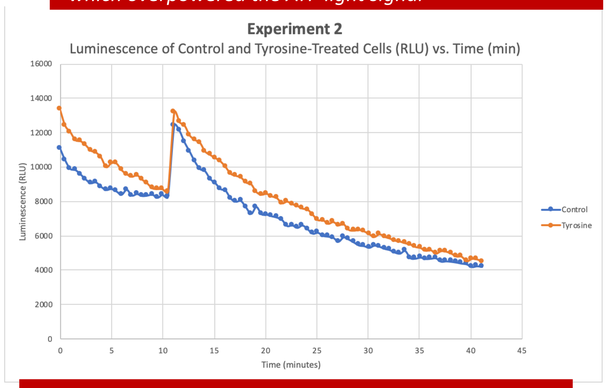


Figure 3 *shows the adjusted results, showing a RLU increase when KCl was added to the control and tyrosine-treated cells, with the tyrosine cells showing more ATP productivity overall*

Conclusion and Future Directions

- Based off our results, we are able to determine that by adding tyrosine to the cells, there is an increase in ATP release. KCl must be added very carefully due to the fact that it may overpower the cells if it is too much and if there are too many cells, the KCl is not able to react. Future work we will be looking at the overall increase of ATP in the cells versus just the ATP released upon addition of the KCl. Additionally, we hope to test substances such as L-theanine and caffeine in combination with tyrosine so see their affects on the cells' ATP productivity. This data is important because it relates to our hypothesis and goal of finding an alternative to increase brain cognition, along with attention span. The increase of ATP shows the potential ability of tyrosine to do exactly that. Because ATP assists in neurotransmission, an increase in ATP production can be correlated with an increase in overall cognitive function and efficiency.

References

Deijen J, Wientjes C, Vullinghs H, Cloin P, Langefeld J. Tyrosine improves cognitive performance and reduces blood pressure in cadets after one week of a combat training course. Brain Research Bulletin. 1999;48(2):203–209.

Hase A, Jung SE, Rot MAH. Behavioral and cognitive effects of tyrosine intake in healthy human adults. Pharmacology Biochemistry and Behavior. 2015;133:1–6.

Imamura H., Huynh K.P., Togawa H., Saito K., Iino R., Kato-Yamada Y., Nagai T., and Noji H. Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators. PNAS. 2009. 106 (37): 15651-15656.

Ostrom R.S., Gregorian C., Insel P.A. Cellular Release of and Response to ATP as Key Determinants of the Set-Point of Signal Transduction Pathways. Journal of Biological Chemistry. 2000. 275: 11735-11739.