Localizing adenosine and dopamine receptors in spinal thoracic sympathetic motor neurons

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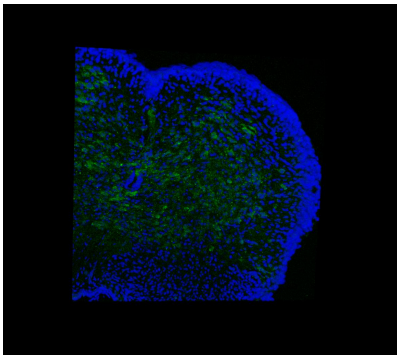
Purpose: Adenosine and dopamine are neurotransmitters which interact to control many bodily functions including sleep and locomotion. Studies have found their receptors (specifically A1 and D1) are localized within somatic motor neurons (MN) in the lumbar spinal cord, forming heteromers that regulate MN firing activity (Rivera-Oliver et al. 2018). These A1-D1 heteromers, however, have not been confirmed in the thoracic spinal cord where sympathetic (autonomic) MNs reside. We are specifically looking in this location because of its importance in controlling fundamental processes that involve major organ systems such as the heart, lungs and gastrointestinal tract. Neural information from the autonomic system travels through a small percentage of dorsal root ganglia neurons in the thoracolumbar spinal regions and preganglionic MN mostly located in the thoracic part of the spinal cord (Zimmerman et al. 2012). Confirming the colocalization of A1-D1 receptor heteromers within thoracic sympathetic preganglionic MN, could provide better understanding of the control of autonomic MN activity in the human body. This study aims to confirm this localization of A1 receptors (A1R) and D1 receptors (D1R) in thoracic spinal cord sympathetic MNs via immunochemistry.

Methods: Mice (P1-P6) were euthanized via rapid decapitation before undergoing ventral laminectomy, which exposed the thoracic spinal cord. Once removed, the cords were fixed and cryoprotected. Then, to process different segments of the thoracic spinal cord, the cords were sliced with a vibrating microtome. Primary antibodies against the A1 and D1 receptors were then incubated in the tissue of the slices followed by secondary antibodies. These secondary antibodies were coupled with different fluorescent markers (A1 = 594 or Red , D1 = 488 or Green), which were then viewed with confocal microscopy.

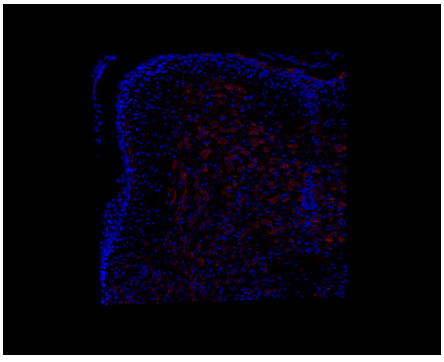
Results: We have evidence for both A1R and D1R staining within the ventral medial and ventral lateral areas of the thoracic spinal cord where MNs reside. Our results support the possibility of A1R and D1R colocalization within the thoracic spinal cord, suggesting A1-D1 heteromers play a role in the regulation of autonomic MN function. Moving forward, we will continue to use immunohistochemical methods to target these A1 and D1 receptors simultaneously in order to see possible overlap under the confocal microscope, which would indicate A1R and D1R colocalization.

Graphs/images/figures: Red = A1R, Green = D1R, Blue = Cell Nucleus



(*Left*) 06-10-21. P1 Slice 3 Z-Stack image 20X left side D1R-488-DAPI.

(*RIGHT*) 06-18-21. P6 Slice 1 Z-stack image 20X right side A1R-594+DAPI.



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References: Rivera-Oliver et al. (2018). Adenosine A1-Dopamine D1 receptor Heteromers control the excitability of the SPINAL MOTONEURON. *Molecular Neurobiology*, *56*(2), 797–811. https://doi.org/10.1007/s12035-018-1120-y
Zimmerman, A. L., Sawchuk, M., & Hochman, S. (2012). Monoaminergic modulation of Spinal Viscero-sympathetic function in the Neonatal Mouse thoracic spinal cord. *PLoS ONE*, *7*(11). https://doi.org/10.1371/journal.pone.0047213