Sex Differences in the Expression of CGRP in C2/A2 Norepinephrine Neurons

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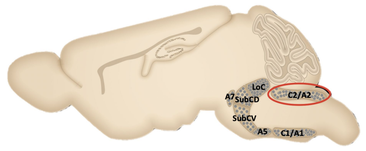
Overview

We used a dual immunohistochemistry approach to evaluate sex differences in the expression of CGRP in the cell bodies of C2/A2 NE neurons of mice. We hypothesized that female mice would have higher expression of CGRP in NE cell bodies of the C2/A2 region, but found that there were no significant sex differences in this region.

Introduction

Norepinephrine, among other functions, raises blood pressure in the vascular system through the sympathetic nervous system. This can go awry with hypertension leading to 410,000 American deaths per year. Preeclampsia, a common pregnancy related condition, is caused by high blood pressure, and can be fatal. Recent research has shown calcitonin gene related peptide (CGRP) to mitigate these effects, protecting cells from norepinephrine induced apoptosis. We hypothesize that there will be sex differences of expression in the A2 region, with females exhibiting higher levels of CGRP than males.



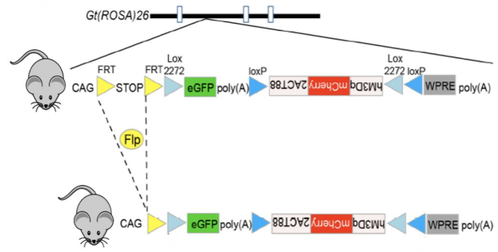
CGRP is an amino acid peptide. Research has been conducted that shows that CGRP is widespread throughout the central and peripheral nervous system, especially in the areas of noradrenergic concentration.[1] CGRP lowers blood pressure following a sympathetic response, and protects cerebrovascular beds.[2] In the periphery, CGRP inhibited electrical stimulation induced contraction of the vas deferens in the rat, limiting the effects of norepinephrine.[3] This suggests that CGRP can have effects outside of the CNS.

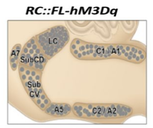
Experimental Outline

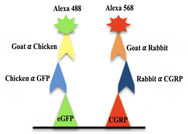
Transgenic mice contain Dbh::Flpo; R/C::FL-hM3Dq so all NE neurons express eGFP → Double immunofluorescence labeling of eGFP in green and CGRP in red → Calculate fluorescence values of CGRP expression in A2 NE cell bodies

Methods

Dbh driven Flp recognizes FRT sites and excises the stop cassette in NE neurons resulting in GFP expression in all NE neurons:

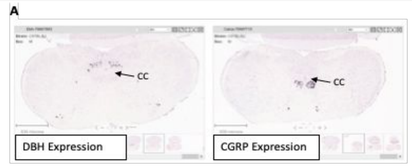


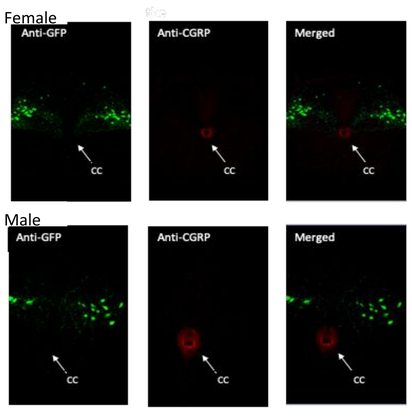




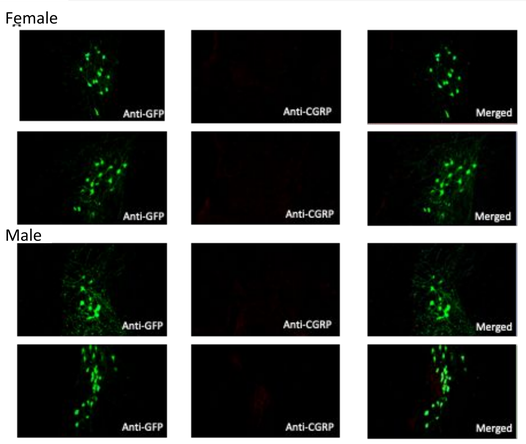
Double immunofluorescence labeling was used to identify eGFP (green) and CGRP (red) expression to evaluate if CGRP is expressed in NE neuron cell bodies in the C2/A2 region.

Double Immunofluorescence of GFP and CGRP in C2/A2

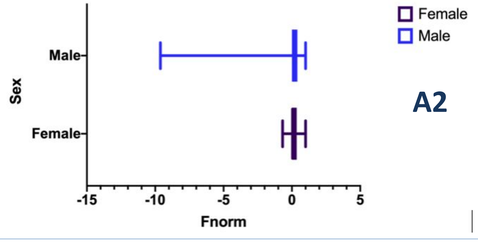




The A2 subpopulation resides above the cc bilaterally in the Allen Mouse Brain Atlas, while CGRP expression was located below the cc. Our immunohistochemistry experiment shows CGRP expression diffusely throughout the section, but mostly prominently around the border of the cc. A2 NE neurons are shown in green.



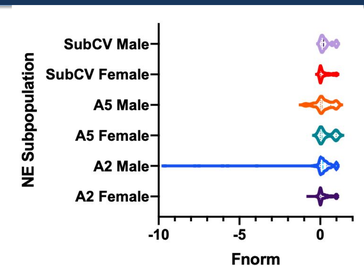
Neither female nor male specimen showed co-localization of CGRP in NE neurons of the A2 anatomical subpopulation.



Statistical Analysis

A2	Female	Male
Number of values	169	138
Minimum	-0.6779	-9.617
25% Percentile	-4.360e-005	0.001050
Median	0.04689	0.1671
75% Percentile	0.3556	0.4024
Maximum	1.000	1.000
Range	1.678	10.62
Mean	0.2196	-0.03155
Std. Deviation	0.3608	1.525
Std. Error of Mean	0.02775	0.1298

After image analysis of male and female A2 NE neurons, no significant difference was found in CGRP expression.



A significant difference was found in CGRP expression in males between A2 and SubCV. All other statistical analysis showed no significance.

Conclusions and Future Directions

Our findings did not indicate any sex-differing expression of CGRP in NE neurons. Though we were not able to present novel results, our study indicates that visual analysis of CGRP in the A2 region is limited by their locality in the cell. CGRP in A2 catecholaminergic cells are primarily located on the axon, which could skew results if these regions of the cell are not stained well for GFP, or this fact is not taken into account in the imaging analysis.[4] Future research would find more significant, and efficient, results regarding sex-differing expression of genes within noradrenergic regions such as A2 through the use of transcriptional analysis. Additionally, future research should focus on expression of the CGRP receptor, rather than the CGRP peptide as the axons of A2 NE neurons project towards high concentrations of CGRP in the cc, suggesting that they may be synaptic partners.

Acknowledgements

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