The Effect of RASD1 G-signaling protein on Atrial Natriuretic Factor Expression

Maria Fam[1]; Dr. Adolfo de Bold[2,3]

[1]Faculty of Health Sciences, University of Ottawa. [2]Cardiovascular Endocrinology Laboratory, University of Ottawa Heart Institute, University of Ottawa, Ottawa, Ontario, Canada.[3] Department of Pathology and Laboratory Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada

Introduction

Cells of the atrial granules of the endocrine heart excrete Atrial Natriuretic Factor (ANF) in response to increased load[1]. ANF is a protein hormone. ANF contributes to homeostasis, the state of equilibrium of the body, through the modulation of blood pressure.

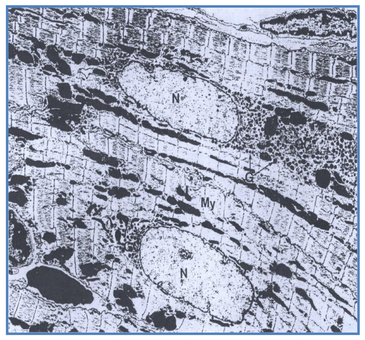
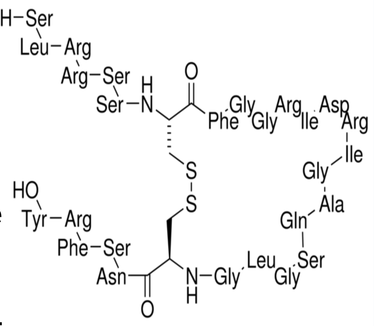


Figure 1 (Left): Electron Microscopy of Atrial Granules [1]. **Figure 2 (Below):** Peptide Sequence of ANF Hormone [2].



As a result, renin secretion via the kidney cells is inhibited.
This decreases the production of aldosterone by the adrenal cortex.
Aldosterone is responsible for sodium and water retention.
Altogether, this system is known as the renin-angiotensin-aldosterone pathway. If the amount of aldosterone is ultimately decreased in response to ANF secretion, then excess sodium and chloride will be excreted via the urine. Vasodilation will occur due to an osmotic response.

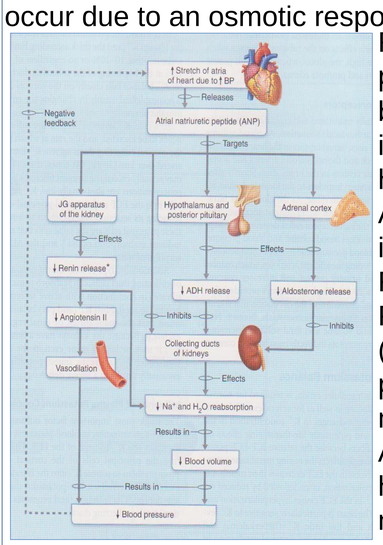


Figure 3: Effects of ANF on body including the renin-angiotensin-aldosterone pathway**[3]**

Ras dexamethasone-induced protein 1 (RASD1) has recently been discovered in humans yet its function is still unknown. It is hypothesized that it may impact ANF secretion in the body. In implementing RNA extraction, Reverse Transcription Polymerase Chain Reaction (PCR) , and Real Time PCR procedures, we are able to monitor the impact of RASD1 on ANF expression of HL-1 cells and hearts from RASD1 knockout mice. This procedure will be repeated on cells along with organs from animals; the events of a cell are not always reflected in that of an animal.

For example, during meiosis, we cannot identfiy these processes looking at animal tissues. For this reason, it is important to observe events on a cellular level as well as at an organismal level.

In observing the effect of RASD1 on ANF expression, one can gain further insight on the regulating factors of ANF and, in future, take advantage of this discovery for potential health benefits.

Hypothesis

A significant relation exists between RASD1 and ANF. An increase in RASD1 activation represents a decrease in ANF expression.

Methodology

1. HL-1 Atrial Cells (HPRT, Negative Control, No Treatment, RASD1 Cell Knockdown).
2. Wild-Type Hearts and Knockout Hearts from Mice

RNA Extraction: RNA was extracted using the TRIzol® Reagent (Life Technologies, Carlsbad, CA, United States)

cDNA Production: Reverse Transcription of RNA samples using the Transcriptor first-strand cDNA synthesis kit (Roche Diagnostics, Laval, QC, Canada)

Real-Time PCR: using LightCycler 480 SYBR Green 1 Master following associated procedure (Roche Diagnostics). The Results were processed and analyzed using Roche LightCycler 480 relative quantification software.

Results

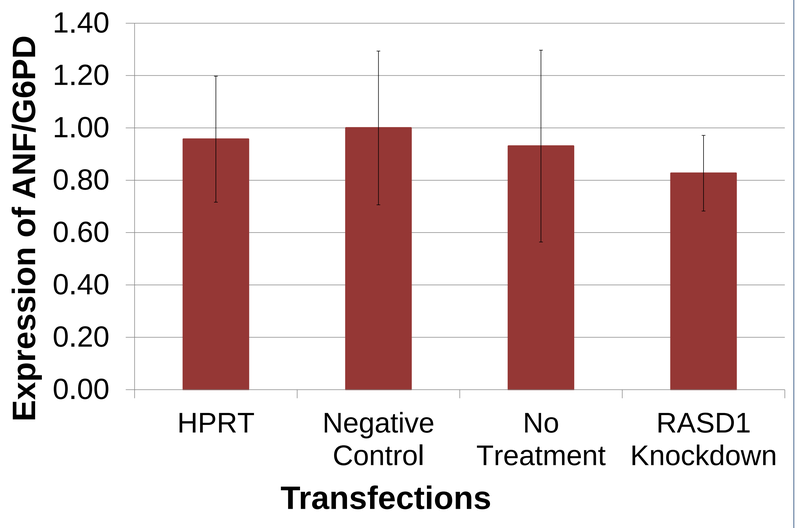


Figure 1: Real Time PCR Expression Values of ANF HL-1 Cells. Values are mean ± SEM, HPRT: n=2; Negative Control: n=3; No treatment: n=3; RASD1 Knockdown: n=3.

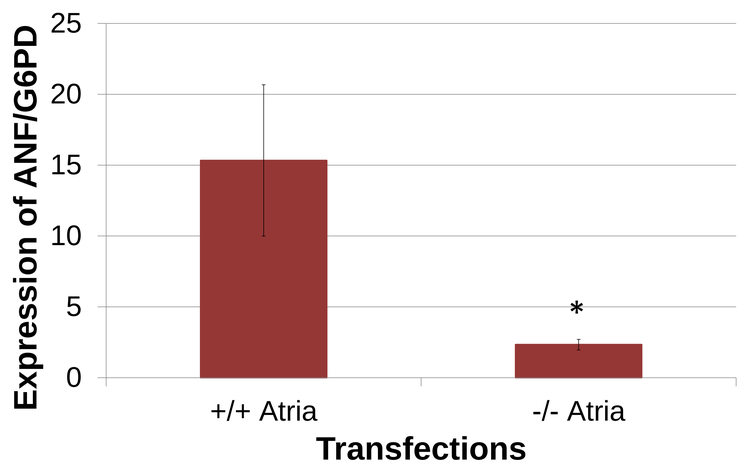


Figure 2: Real Time PCR Expression Values of ANF in Atria of Mice. Values are mean ± SEM; Wildtype: n=6; Knockout: n=6; *p<0.05 vs Knockout RASD1.

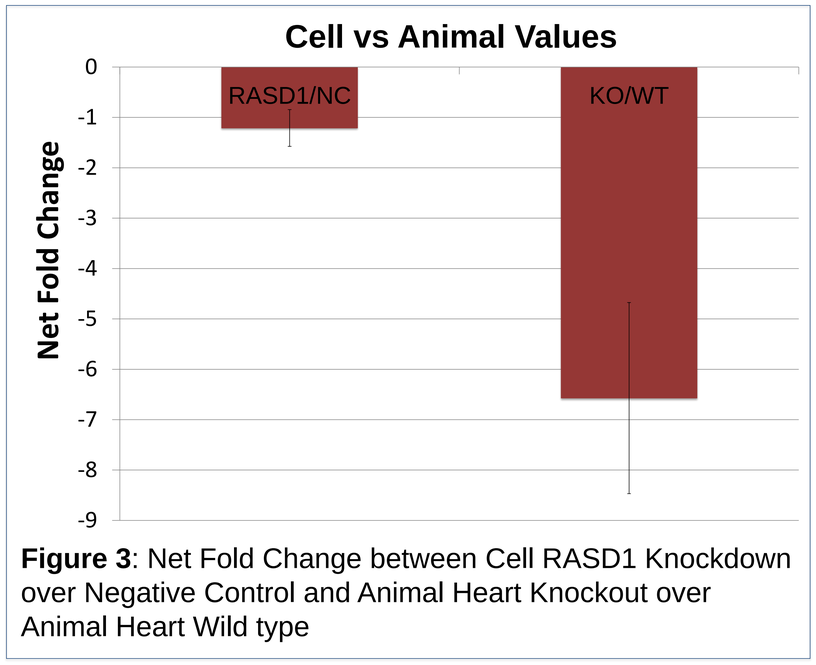


Figure 3: Net Fold Change between Cell RASD1 Knockdown over Negative Control and Animal Heart Knockout over Animal Heart Wild type

Conclusion

- Figure 1 results are not significant. Figure 2 results have a $p<0.05$ meaning they are statistically significant.
- We reject the hypothesis. Although there is a significant relation between RASD1 and ANF, the described relationship is incorrect. An increase in RASD1 activation represents an increase in ANF expression only in animals; not in cells.
- RASD1 G protein is therefore a negative modulator of ANF expression in animals.
- The results reflect what happens in individual cells does not necessarily imitate what happens in the overall organism [4]
- This beneficial discovery can now be utilized to further understand prevention strategies for cardiovascular diseases including heart failure and cardiac hypertrophy.
- With further research on RASD1's interactions with other molecules and enzymes, one can further understand it's role as a negative modulator of ANF**[4]**

References & Acknowledgements

Works Cited: [1] De Bold A.J. , Bruneau B.J. . Natriuretic Peptides. Compr Physiol 2011, Supplement 22: Handbook of Physiology, The Endocrine System, Endocrine Regulation of Water and Electrolyte Balance: 377-409. First published in print 2000. doi: 10.1002/cphy.cp070310. **[2]** Sigma Aldrich Co. (2013). Atrial Natriuretic Peptide rat. Retrieved from: http://www.sigmaaldrich.com/catalog/product/sigma/A8208?lang=en&region=CA **[3]** Marieb, E. N., Hoehn K. (2010). Fluid, Electrolyte, and Acid-Base Balance (8th Ed.), *Human Anatomy & Physiology* (pp.1005). San Francisco, CA. **[4]** McGrath MF, Ogawa T, de Bold A.J. Ras dexamethasone-induced protein 1 is a modulator of hormone secretion in the volume overloaded heart. *American journal of Physiology: Heart and circulatory physiology*. 302(9)):H1826-37. doi: 10.1152/ajpheart.01085.2011. **[5]** Zhegunov, Genadiy. (2012). *The Dual Nature of Life* (pp. 73-80). Springer Berlin Heidelberg

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