Exploring dopamine receptor and beta-2-adrenergic receptor signaling in modulating the expression of MMPs and TIMPs in human cardiac fibroblasts

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INTRODUCTION

- Beta-blockers are some of the most prescribed drugs today, most notably among 22.3% of the geriatric population in the United States.[1]
- These drugs are used to inhibit beta-adrenergic signaling that consequently lower heart rate and contractility, culminating in lower blood pressure.[2]
- Dopamine has long been shown to be involved in cardiovascular disease, and its receptors in cardiac tissue have been pharmacological targets for congestive heart failure.[3]
- Our lab has previously highlighted the importance of dopamine receptor 3 (D3R) and its role in fibroblast proliferation during wound healing.[4]
- This project seeks to understand whether there is cross-talk between β2 and D1 receptors, as well as any effect on the expression of matrix metalloproteinases (MMPs) and tissue inhibitor metalloproteinases.[5]
- Understanding the interaction between these two signaling pathways can provide deeper insights into the cardiac remodeling process following physiological stress.

MATERIALS & METHODS

Cell Culture: Cryopreserved human cardiac fibroblasts (hCFB) (C12375) from PromoCell were centrifuged, mixed and suspended in media and serum, incubated at 37°C and 5% CO_2 in cell culture flasks. Cells were cultured from passages 3-7. Treatments were designed using dopamine (DA), D1R antagonist SCH 39166 hydrobromide (SCH), D3R agonist pramipexole dihydrochloride (PPX), and β2 agonist isoproterenol.

Real Time quantitative PCR: RNA was extracted and isolated from control and treatment groups for subsequent analysis with RT-qPCR. Control groups with dopamine were used as the standard of comparison for statistical analysis.

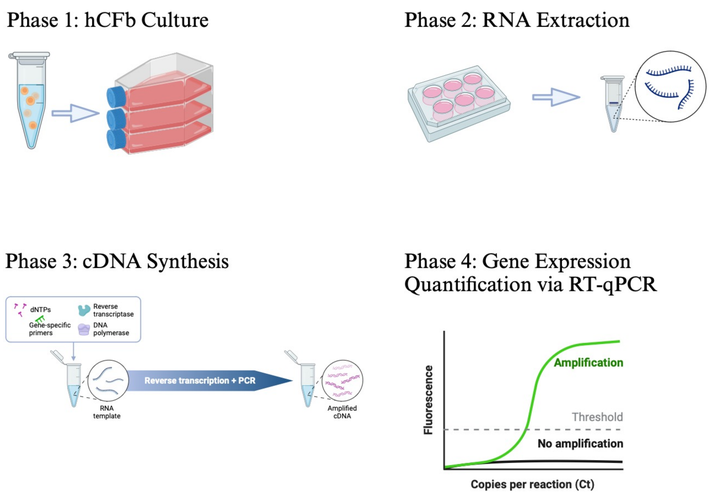

Figure 1. *Schematic of experimental design.*

RESULTS

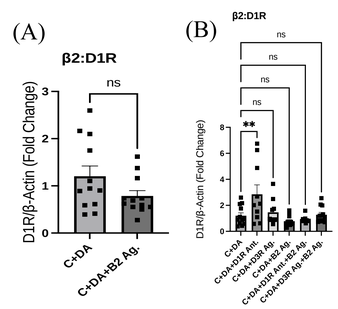

Figure 2: *β2 stimulation on D1R gene expression (β2:D1R). (A) Comparing the gene expression of D1R between control+dopamine and β2 agonist . β2 agonist had no significant alterations in DRD1 gene expression. (B) SCH treatment increased expression of DRD1 (*p < 0.01). Other treatment groups showed no significant differences as compared to the control.*

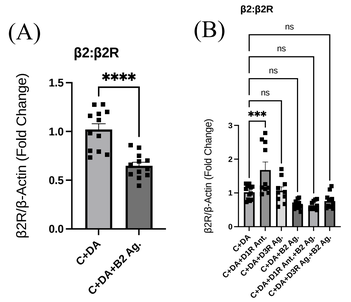

Figure 3: *β2 stimulation on β2R gene expression (β2: β2R). (A) Comparing the gene expression of β2R between control+dopamine and β2 agonist . β2 agonist had significant decrease in β2R gene expression (*p < 0.0001). (B) SCH treatment increased expression of β2R (*p < 0.001). Other treatment groups showed no significant differences as compared to the control.*

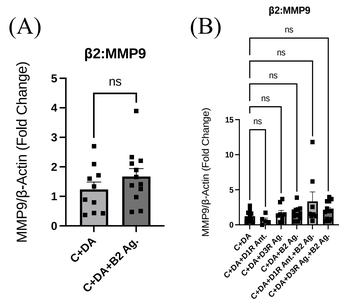

Figure 4: *β2 stimulation on MMP9 gene expression (β2:MMP9). (A) Comparing the gene expression of MMP9 between control+dopamine and β2 agonist . β2 agonist had no significant decrease in MMP9 gene expression. (B) Treatment groups showed no significant differences as compared to the control.*

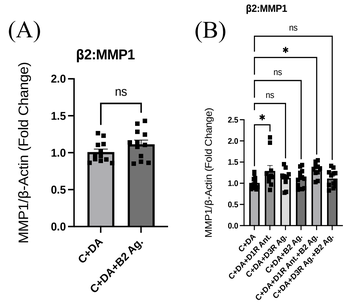

Figure 5: *β2 stimulation on MMP1 gene expression (β2:MMP1). (A) Comparing the gene expression of MMP1 between control+dopamine and β2 agonist . β2 agonist had no significant decrease in MMP1 gene expression. (B) SCH treatment and SCH+ β2 agonist both increased expression of MMP1 (*p < 0.05). Other treatment groups showed no significant differences as compared to the control.*

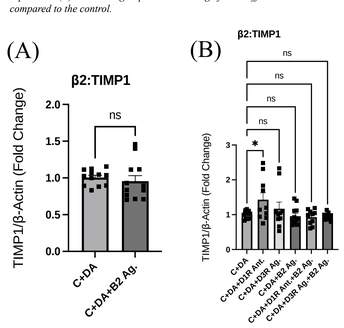

Figure 6: *β2 stimulation on TIMP1 gene expression (β2:TIMP1). (A) Comparing the gene expression of TIMP1 between control+dopamine and β2 agonist . β2 agonist had no significant decrease in MMP1 gene expression. (B) SCH treatment showed increased expression of TIMP1 (*p < 0.05). Other treatment groups showed no significant differences as compared to the control.*

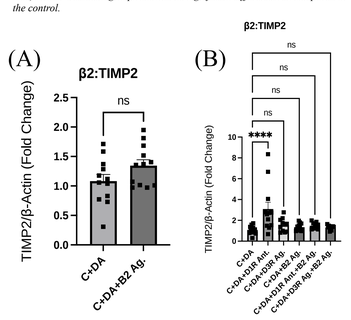

Figure 7: *β2 stimulation on TIMP2 gene expression (β2:TIMP2). (A) Comparing the gene expression of TIMP2 between control+dopamine and β2 agonist . β2 agonist had no significant decrease in TIMP2 gene expression. (B) SCH treatment showed increased expression of TIMP2 (*p < 0.0001). Other treatment groups showed no significant differences as compared to the control.*

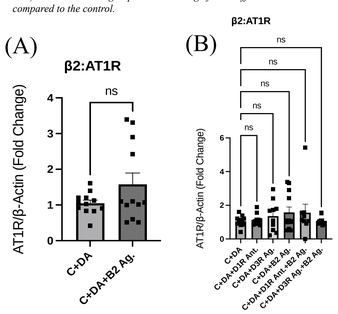

Figure 8: *β2 stimulation on AT1R gene expression (β2:AT1R). (A) Comparing the gene expression of AT1R between control+dopamine and β2 agonist . β2 agonist had no significant decrease in AT1R gene expression. (B) Treatment groups showed no significant differences as compared to the control.*

Summary of Results:

- Presence of dopamine lead to β2 agonist decreasing expression of β2R.
- β2 agonist showed marginal increase in MMP1 expression when in combination with D1R antagonist, as compared to D1R antagonist alone.
- D1R antagonist increased expression of D1R, β2R, MMP1, TIMP1, and TIMP2 genes.

DISCUSSION

- β2 levels decreased under a negative feedback system when exposed to isoproterenol, potentially serving as a mechanism preventing tachycardia.
- D1R antagonist has been shown to behave similarly to angiotensin II and β2 regarding hypertensive activity. This experiment showed D1R antagonist increases levels of β2 to maintain contractility properties, to which its antagonistic properties might help prevent ventricular arrythmia. This suggests some cross-talk between the two receptors.
- Tissue inhibitor metalloproteinases (TIMPs) were upregulated by D1R antagonist. D1R antagonist's influence on MMP1 upregulation was insignificantly increased by the presence of isoproterenol.
- **D1R antagonist is a profound stimulator of multiple genes involved in the structure, function, and maintenance of the cardiac extracellular matrix.**

FUTURE DIRECTIONS

- Repeat RT-qPCR experiments to confirm results and resolve discrepancies in data.
- Conduct enzyme-linked immunosorbent assays (ELISA) to determine protein expression of MMP1 and MMP9.
- Further postulate signaling pathways of SCH involving upregulation of MMPs and TIMPs, as well as potential cross-talk with other adrenergic receptors.

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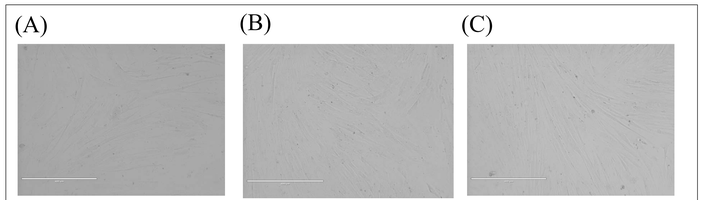

Figure 9. *Human cardiac fibroblasts, passage 7.*