Role of G Protein-Coupled Receptor kinase 5 in Animal Model of Cardiac Hypertrophy

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Abstract

Background: G protein couple receptor kinase 2 and 5 (GRK2 and GRK5) are the main negative regulator for β-adrenoreceptor activation, which play an important role in cardiac function and contractility. Up-regulation in GRK2 levels has been found play an important role in cardiac hypertrophy development. However, little is known about whether GRK5 modulates Isoproterenol induced cardiac hypertrophy and the change in protein level of GRK5 in animal model of cardiac hypertrophy is not well elucidated yet. **Aim**: This research aims to investigate the change in GRK5 protein expression in Isoproterenol -induced cardiac hypertrophy animal model. **Method**: Cardiac hypertrophy was induced in rats by daily Intraperitoneal injection of Isoproterenol (5mg/kg/day). After three weeks, cardiac hypertrophy biomarkers were assessed. GRK5 protein expression were detected using western blotting and immunohistochemistry techniques. **Results**: Key findings data showed that serum creatinine, troponin T and heart weight/ body weight ratio significantly increased in cardiac hypertrophic rats. Furthermore, a significant up-regulation in GRK5 protein expression was observed in hypertrophic myocardium tissue. **Conclusion:** Our research yielded promising results verifying a strong link between animal model cardiac hypertrophy and an over expression of GRK5. Our results suggest that over-expression of GRK5 in animal model of cardiac hypertrophy might play an important role in pathological cardiac hypertrophy development. Furthermore, over-expressed GRK5 could be suggested as new marker to investigate cardiac hypertrophy development or as potential therapeutics target.

Introduction

Cardiac hypertrophy is defined as an abnormal enlargement or thickening of the heart mainly caused by an increase in the size of cardiomyocytes.

Studies report an association linking pathological cardiac hypertrophy development and an increase in numerous intracellular signaling molecules. Most prominently, the G protein coupled receptor kinases (GRKs) have been observed in cardiac-related medical condition, most likely due to their prominence within the myocardium. Importantly, GRK5 can act as a class II HDAC kinase in cardiomyocyte nuclei, indicating that GRK5 plays an important role in pathological cardiac hypertrophy in a non-canonical GPCR-independent manner [1].

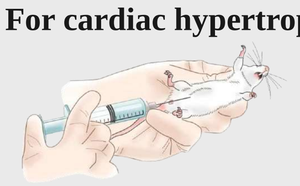
Therefore, we conducted this research shedding light specifically on the expression of GRK5 in animal model of cardiac hypertrophy.

GRK5 STRUCTURE

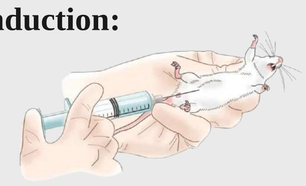
Figure- 1: GRK5 functions via phosphorylating and terminating the stimulation of GRK bound agonist [2].

Materials and Methods

For cardiac hypertrophy induction:



Cardiac hypertrophy Was induced in 13 rats via daily intraperitoneal Injections of Isoproterenol (5mg/kg/day) [3]



7 remaining rats served as our control group via daily intraperitoneal injections of normal saline

For evaluation of experimental cardiac hypertrophy:

- ❑3 weeks post induction, the heart weight/ body weight ratio was calculated.
- ❑A biochemical assay measuring the serum Creatinine Kinase MB and Troponin T level was conducted.
- ❑Myocardium tissues were subjected to both Heamatoxylin and eosin and Massson's trichome.

For investigation of GRK5 expression:

Left ventricle tissues were subjected to western blotting and immunohistochemistry using a specific antibody against GRK5. Ethics reference number for this study is KSU-SE-20-25.

References

1- Leinweber K., Cardiovascular Research, 2005.
2- Traynham C., Journal of molecular and cellular cardiology, 2016.
3- Chowdhury D., PubMed, 2013.

Results

CARDIAC HYPERTROPHY BIO MARKERS

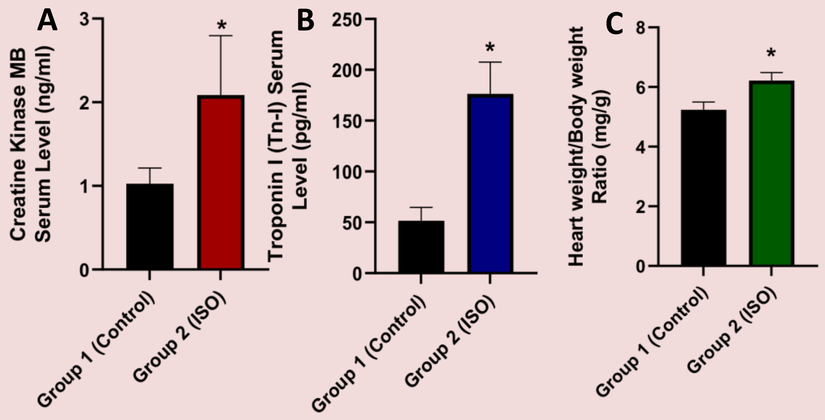


Figure-2: Increase in serum Creatinine MB, Troponin T levels and heart weight/body weight ratio in Isoproterenol-induced cardiac hypertrophy.

Effect of Isoproterenol on cardiac hypertrophy bio-markers including serum Creatinine MB **(A)**, Troponin T **(B)** levels, and on heart weight/body weight ratio **(C)** confirming induction of cardiac hypertrophy. Data is presented as mean ± SED. *P≤0.05 compared to normal control group, using unpaired t-test.

HISTOPATHOLOGICAL CHANGES IN CARDIAC HYPERTROPHY

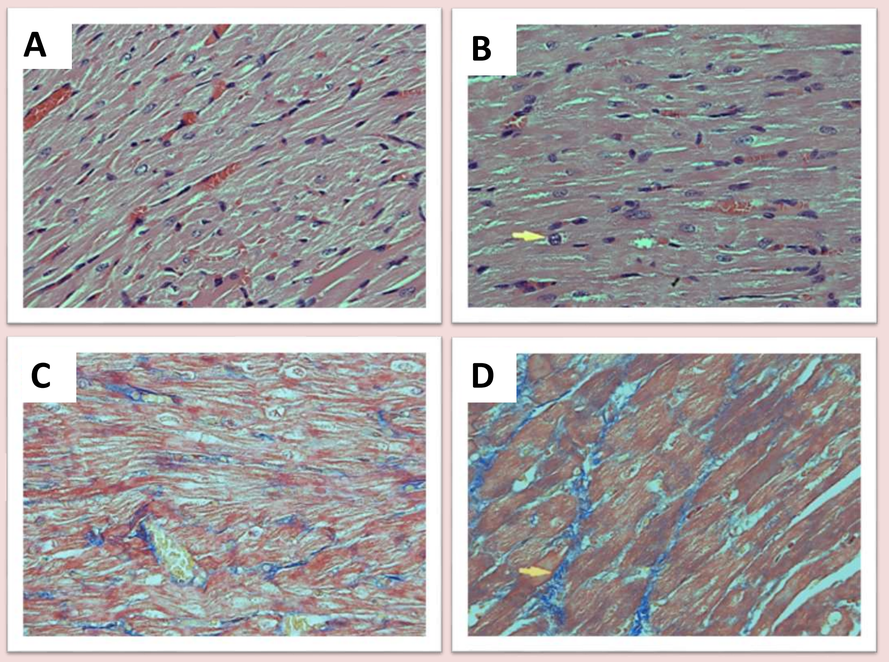


Figure- 3: Haematoxylin and eosin staining for cardiac morphology and Masson's trichome staining for cardiac fibrosis.

(A) Section from the ventricle obtained from the normal control group. Note the normal myocardial fibers and regular nuclei. **(B)** H&E stained section from Isoproterenol treated rats. Myocardium showing residual abnormal fibers showing enlarged nuclei (arrow) indicative of ventricular hypertrophy H&E stain x400. **(C)** photomicrograph showing normal distribution of collagen (blue fibers) within the myocardium of control group. **(D)** section of Isoproterenol treated rat's myocardium, note the presence of increased blue stained collagen fibers (arrow) indicative of mild ischemic changes and ventricular hypertrophy. Masson's trichrome special stain x400.

UP-REGULATION OF GRK5 IN CARDIAC HYPERTROPHY

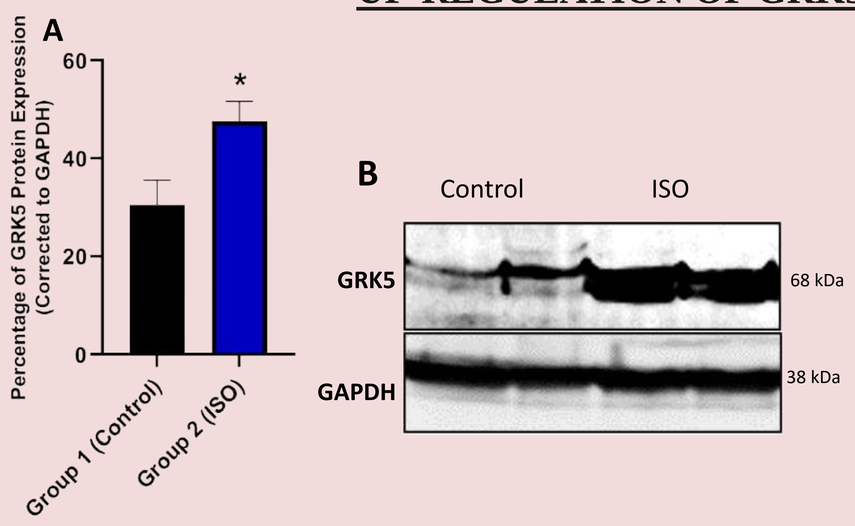


Figure- 4: Over-expression of GRK5 in Isoproterenol-induced cardiac hypertrophy using immunoblotting.

(A) Bar graph showing the changes in GRK5 protein expression. Band density wasquantified by odyssey licor analysis program and normalized to GAPDH housekeeping gene. **(B)** Representative blot showing showing the changes in GRK5 protein expression. Data is presented as mean ± SED. *P≤0.05 compared to normal control group, using unpaired t-test.**]**

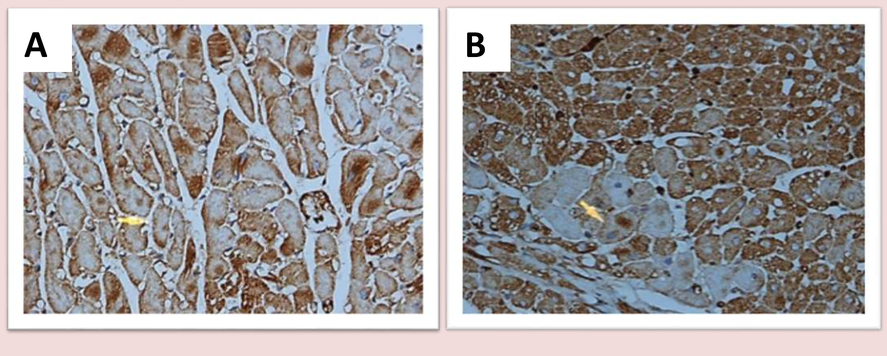


Figure- 5: Over-expression of GRK5 in Isoproterenol-induced cardiac hypertrophy using immunohistochemistry.

(A) Photomicrograph representing a transverse section for the control myocardium stained with antibody against GRK5. Note the variable negative and positive cytoplasmic staining which indicates variable absent (arrow) or weak expression.

(B) Photomicrograph representing a section from the myocardium of Isoproterenol treated group, note the presence of strong cytoplasmic brown stating indicative of over expression of of GRK5, IHC stain GRK5 x400.

Conclusion

In conclusion, this research positively confirms and documents data proving an increased expression of GRK5 in animal model of cardiac hypertrophy. This serves as a solid foundation for utilization of this data in targeted treatment for cardiac-related medical conditions or as potential medical bio markers.

Acknowledgments

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