Biased Agonist Modulation of Carvedilol & Metoprolol in β-arrestin pathway

Luay Boulahouache*[1], Salvi Kumar*[2], Dr. Arfaxad Reyes Alcaraz, and Dr. Bradley McConnell
University of Houston College of Pharmacy, Houston, TX. United States of America

Contact Information:
(1) **Luay Boulahouache**
University of Houston
Email: lboulahouache@uh.edu

(2) **Salvi Kumar**
University of Houston
Email: skumar17@uh.edu

*:These co-first authors equally contributed to this study.

ABSTRACT

Carvedilol and Metoprolol are first line medications to treat heart failure. Structural complementation assay based on NanoBiT technology was used in this work to monitor the pharmacology in real time of two typical drugs to treat heart failure. A higher antagonistic effect was observed in the case of carvedilol in β-arrestin recruitment as well as receptor internalization. We conclude that β-arrestin signaling might be a valid therapeutic approach to treat heart failure.

BACKGROUND

- About 6.5 million adults in the United States have congestive heart failure (CHF), the impaired ability of the heart to efficiently pump blood.
- Chronic β2-AR stimulation, which occurs in CHF, results in reduced contractility due to desensitization by β-arrestins of these receptors.
  - β2-AR is a G protein-coupled receptor (GPCRs), which has been successfully targeted by numerous therapeutics including drugs.
- Therapeutic drugs like Carvedilol and Metoprolol (known as β-blockers) are combined with ACE inhibitors to significantly reduce mortality, decrease sudden death, and improve symptoms in patients with HF.[1]
  - Carvedilol has shown to better reduce mortality rate relative to metoprolol.
  - The reason for such discrepancies may be explained via "biased agonist modulation."

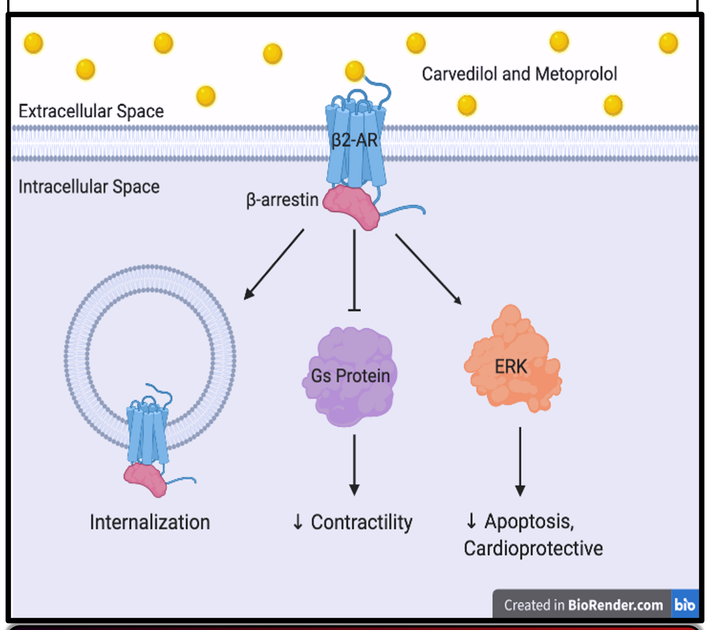

Figure 1. Metoprolol and Carvedilol (β-blockers) stimulating the β-arrestin pathway.

OBJECTIVE

Understand the β-arrestin signaling pathway between carvedilol and metoprolol.

The overall aim of this work is to accelerate drug discovery and drug development by better understanding the role of biased agonist signaling within current therapeutic drugs for CHF.

METHODS

Study design / Inclusion

- HEK293 cells were obtained from the American Type Culture Collection (ATCC, CRL-1573; Manassas, VA, USA) and were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin G, and 100 µg/ml streptomycin (Invitrogen; Carlsbad, CA, USA).
- Transfection of the two constructs was made using Lipofectamine 2000 (Invitrogen).

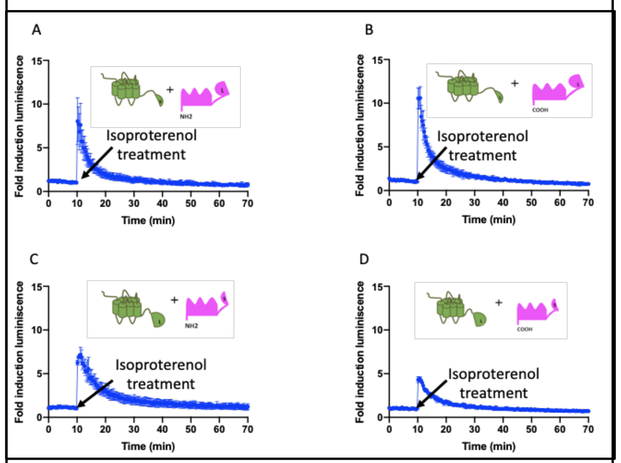

Figure 2. Four different plasmid combinations (β2AR and β-arrestin2) were screened, in order to select the one with the highest luminescent signal for further studies.

- At 24 h post transfection, the medium was aspirated and replaced with 100 µl OPTIMEM, then 25µl substrate (furimazine) was added and once every minute subsequent luminescence measurements were taken for 10 min for signal stabilization. A total of 10 µL ligand was then added to each well and luminescence measurements were recorded immediately and once every minute for 1 h.

RESULTS

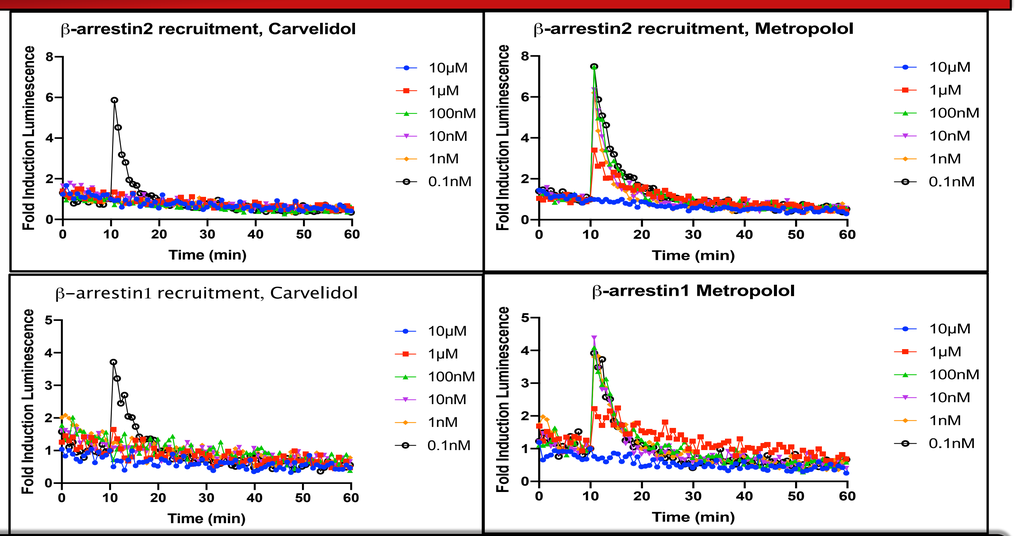

Figure 3. Antagonist effect of Carvedilol and Metoprolol in β-arrestin1/2 recruitment at β2AR in living cardiomyocytes.

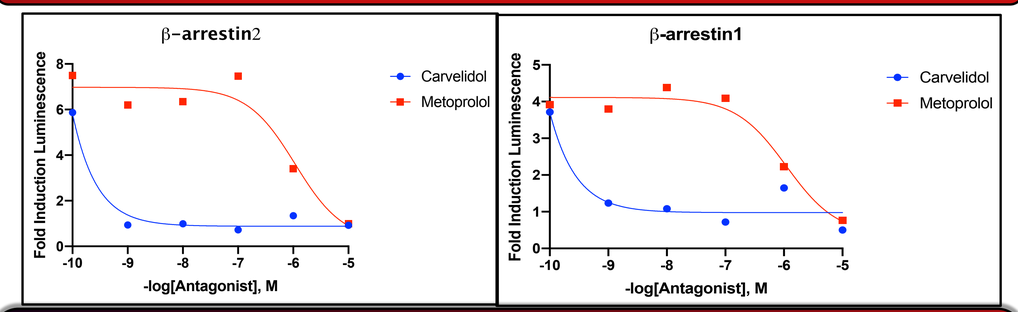

Figure 4. Dose Response Curves of β-arrestin1/2 recruitment in the presence of different concentrations of antagonists. The antagonist was added first and incubated for 30min at 37°C and 5% CO_2. Finally epinephrine was added, and luminescent signal was immediately recorded.

CONCLUSIONS

Carvedilol has higher potency than Metoprolol, as shown by the effectiveness of Carvedilol from blocking β-arrestin signaling which indicates that Carvedilol has a higher affinity to β2AR. Carvedilol administers the same antagonistic effects at a lower dose than Metoprolol, which can reduce possible side effects due to high dosages. Further research of these two β-blockers regarding the selectivity of β-arrestin to Gs signaling should be compared and analyzed along with selectivity of β1-AR compared to β2-AR in β-arrestin signaling.

References

1. Gheorghiade M, Colucci WS, Swedberg K. Beta-blockers in chronic heart failure. Circulation. 2003; 107: 1570–1575.

Acknowledgements

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