A new synthetic peptide regulates hypertrophy in vitro through means of the inhibition of NFκB

Authors:
C Del Giudice¹, D Sorriento², M Ciccarelli³, E Vernieri³, P Campiglia³, B Trimarco¹, G Iaccarino³, ¹Federico II University of Naples - Naples - Italy, ²National Research Council - Naples - Italy, ³University of Salerno - Salerno - Italy,

Topic(s):
Signal transduction - Heart

Citation:
Cardiovascular Research Supplements (2016) 111 (S1), S59

Background: GRK5 is part of the family of G protein coupled receptors kinases which are known to regulate GPCR through phosphorylative events. It has been shown that the amino-terminal region of GRK5 (GRK5-NT) is able to regulate cardiac hypertrophy both in vitro and in vivo, through the inhibition of the transcription factor NFκB, by means of the RH domain. We have identified the potential minimum sequence (10 aa) of RH which is able to inhibit NFκB, and we synthesized a new peptide that mimics this sequence (TAT-RH10).

Purpose: the aim of this study is to identify the minimum effective region of RH, evaluate its effectiveness on NFκB activity in cardiomyoblasts in response to hypertrophic stimuli and evaluate its effectiveness on the regulation of calcium-calmodulin dependent cardiac transcription factors.

Methods: Experiments were performed in cardiomyoblasts (H9C2). Hypertrophy in vitro was induced by chronic stimulation with phenylephrine (Phe 10-5M). Real Time PCR was performed to evaluate gene expression of Atrial Natriuretic Factor (ANF). NFκB signaling was assessed by Western blot (WB).

Results: To verify the effectiveness of TAT-RH10 on NFκB signaling, we evaluated the total levels of IkBa by WB. TAT-RH 10 increased IkBa levels and inhibited the transcriptional activity of NFκB. Thus, TAT-RH 10aa is able to inhibit NFκB signaling in H9c2 in response to a hypertrophic stimulus. To confirm these data, we evaluated the effect of TAT-RH 10 on the expression of the atrial natriuretic factor, ANF, marker of hypertrophy, by Real Time PCR. TAT-RH10 inhibited ANF gene expression both basally and in response to phenylephrine. We then evaluated the effect of TAT-RH10 on the regulation of NFκB dependent phenotypes, such as proliferation and apoptosis. TAT-RH10 inhibited cell proliferation both basally and in response to Phe. Accordingly, TAT-RH10 reduced DNA synthesis both basally and in response to stimulation with Phe. Moreover we evaluated its effects on other cardiac transcription factors, NFAT and GATA-4. TAT-RH is able to regulate NFκB activation but had no effects on NFAT and GATA-4. TAT-RH is specific on NFκB signaling.

Conclusions: In conclusion, we have identified a minimum effective sequence of the RH domain that is able to regulate the transcriptional activity of NFκB in cardiac cells thus leading to the inhibition of NFκB dependent phenotypes in response to hypertrophic stimuli. Further studies will be needed to evaluate the effectiveness of TAT-RH10 in vivo in an animal model of cardiac hypertrophy.