MELANOCORTIN RECEPTOR 5(MC5R) CARDIO-PROTECTION IN HIGH GLUCOSE-INDUCED H9C2HYPERTROPHY

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Introduction: Cardiac hypertrophy is caused by an increased glucose uptake into the cardiac myocytes. This is mostly due to an imbalance of the translocation of GLUT1and GLUT4glucose transporters from intracellular membranes to the cell surface of the myocytes with a GLUT1/GLUT4ratio favoring GLUT1. It is well known that regulation of the glucose homeostasis and insulin sensitivity involves the central melanocortin system, mostly through the hypothalamic proopiomelanocortin (POMC) which is well-established regulator of insulin secretion, glucose utilization, and glucose production. However, scant data exist about the role of peripheral melanocortin peptides or peptidomimetics in this regulation Recently, it has been shown that peripheral α-melanocyte stimulating hormone (α-MSH) promotes glucose uptake in the skeletal muscle via melanocortin receptor 5(MC5R) pathway. Therefore, this study explored the anti-hypertrophic effect of the melanocortin MC5R stimulation in H9c2cardiac myocytes exposed to high glucose.

Materials and methods: H9c2(2-1) cells were exposed to 5.5mM D-glucose (Normal control group, NG); 5.5mM D-glucose + Angiotensin II (1μM, Ang II group), positive control for cardiomyocytes hypertrophy); 33mM D-glucose (High glucose group, HG); 33mM D-glucose + α-MSH (90 pM; HG + α-MSH group); 33mM D-glucose + MC5R agonist PG-901(10^{-10} M), dissolved in PBS (HG + PG-901group); 33mM D-glucose + MC5R antagonist PG-20N (130 nM), dissolved in PBS (HG + PG-20N group). H9c2cardiomyocytes were stimulated with high glucose medium, with or without α-MSH,PG-901, and PG-20N treatment, for 48h, being the 33mM high glucose concentration reported to induce cardiac hypertrophy in H9c2cardiomyocvtes after 2days of exposure. After miR-133a transfection, the following biochemical analysis were performed: cell viability assay, MC5R mRNAs and miR-133a levels determination by qRT-PCR, immunocytochemistry and Western Blot for MC5R, Urotensin II receptor and K_{IR}6.1protein levels assessment, ELISA tests for PI3K activity, plasma membrane GLUT1, GLUT 4and ATP levels determination.

Results: The study shows for the first time an up-regulation of MC5R expression levels in H9c2cardiomyocytes exposed to, compared to cells grown in. Moreover, H9c2cells exposed to high glucose showed a significant reduction in cell viability (-40%), a significant increase in total protein per cell number (+109%), and an increase of the urotensin receptor expression levels as an evidence of cells hypertrophy. The pharmacological stimulation of MC5R with α-MSH of the high glucose exposed H9c2cells increased the cell survival (+50,8%) and reduced the total protein per cell number (-28,2%) with respect to high glucose alone, confirming a reduction of the hypertrophic state as per cell area measurement. Similarly, PG-901significantly increased cell viability (+61,0 %) and reduced total protein per cell number (-40,2%), compared to cells exposed to high glucose alone. Interestingly, the MC5R agonist reduced the GLUT1/GLUT4glucose transporters ratio on the cell membranes exhibited by the hypertrophic H9c2cells and increased the intracellular PI3K activity, mediated by a decrease of the levels of the miRNA miR-133a.

Discussion and conclusions: The beneficial effects of MC5R agonism on the cardiac hypertrophy caused by high glucose was also observed also by echocardiographic evaluations of rats made diabetics with streptozotocin (65mg/kg i.p.). Therefore, the MC5R seems to be a new target in high glucose-induced cardiac myocytes derangements, and an agonism at this receptor can be a strategic tool to reduce these conditions. In vitro, the selective MC5R agonism seems to reduce GLUT1/GLUT4ratio through PI3K activation, mediated by a decrease in miR-133a levels. These evidences open new possibilities for therapeutic interventions through peripheral melanocortin pathways.