Topic 5 – Diabetes, lipids, metabolism – A

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0341

AMPK exerts an insulin-sensitizing effect on cardiac glucose uptake by multiple molecular mechanisms including cytoskeleton reorganization

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Background: Insulin-resistant cardiomyocytes are characterized by a decreased ability of insulin to stimulate glucose uptake. We have previously shown that the activation of AMPK by metformin or phenformin restores insulin-sensitivity in insulin-resistant cardiomyocytes. The aim of our present work is to understand by which molecular mechanisms AMPK exerts its insulin sensitizing effect. In this study we focused on the mTOR/p70S6K pathway and on cytoskeleton reorganization. mTOR/p70S6K, which is known to be inhibited by AMPK, is able to reduce insulin signaling via a negative feedback loop involving serine phosphorylation of IRS-1. On the other hand, cytoskeleton reorganization, which is a known target of AMPK, is responsible for the translocation of the glucose transporter GLUT4 to the plasma membrane.

Methods: Adult rat cardiomyocytes were primary cultured and treated with different agents including insulin, AMPK activator (phenformin), mTOR inhibitor rapamycin and/or actin cytoskeleton inhibitor latrunculin B. Glucose uptake was assessed by detritiation of 2-^{3}H-glucose.

Results: First, we tested if rapamycin was able to mimic AMPK activators. Similarly to phenformin, rapamycin increased the insulin-dependent phosphorylation of Akt involved in the regulation of glucose uptake. Despite the ability of rapamycin to induce this Akt over-phosphorylation, rapamycin was not able to restore the insulin-dependent stimulation of glucose uptake like phenformin did. On the other hand, latrunculin B abolished the insulin-sensitizing action of phenformin on glucose uptake, in insulin-sensitive as well as in insulin-resistant cells.

Conclusions: actin cytoskeleton reorganization but not mTOR/p70S6K, is involved in the insulin-sensitizing effect of AMPK on cardiac glucose uptake. The role played by Small G proteins, known to be involved in the regulation of actin cytoskeleton is under investigation.

0366

A role for focal adhesion kinase in the stimulation of glucose transport in cardiomyocytes

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Background: Stimulation of glucose transport in response to insulin or metabolic stress is an important determinant of cardiomyocytes function and survival, particularly during ischemia-reperfusion episodes. Stimulation of glucose transport is markedly impaired in cardiomyocytes exposed to free fatty acids (FA), despite relative preservation of insulin- or metabolic stress signaling.

Aim: To determine whether Focal Adhesion Kinase (FAK) activity is required for stimulation of glucose transport in cardiomyocytes, and whether FAK downregulation participates in FA-induced impairment of glucose transport stimulation.

Methods: Glucose transport, measured in isolated cardiomyocytes, was acutely stimulated either by insulin treatment, or by metabolic inhibition with oligomycin resulting in AMP-activated kinase (AMPK) activation. FAK activity was inhibited pharmacologically by preincubation with PF-573,228 (PF). FAK activity was assessed from its autophosphorylation on residue Y397, and from the phosphorylation of paxillin on Y118.

Results: Y397 FAK phosphorylation was reduced in cardiomyocytes chronically exposed to FA. Preincubation with PF prior to determination of glucose transport resulted in a significant reduction of oligomycin-stimulated glucose transport, with a modest reduction in insulin-stimulated glucose transport. Insulin and AMPK signaling was unaffected by PF preincubation. Intriguingly metabolic stress provoked Y397 FAK dephosphorylation and deactivation, as evidenced by a concomitant reduction in Y118 paxillin phosphorylation.

Conclusions: stimulation of glucose transport by insulin or metabolic stress in cardiomyocytes requires FAK activity prior to stimulation; FAK activity is however acutely reduced during metabolic stress. The chronic reduction of FAK activity in cardiomyocytes exposed to FFA partially explains the loss of glucose transport responsiveness to insulin or metabolic inhibition.

0140

AKT-mediated cardioprotective effects of aldosterone in type 2 diabetic mice

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Purpose: Studies have shown that aldosterone would have angiogenic effects, and therefore would be beneficial in the context of cardiovascular diseases. We thus investigated the potential involvement of aldosterone in triggering a cardiac angiogenic response in the context of type-2 diabetes, and the molecular pathways involved.

Procedure: 3 week-old male mice, overexpressing aldosterone-synthase (AS), and their controls littermates (WT) were fed with a standard or high fat, high sucrose (HFHS) diet. After 6 months of diet, mice were sacrificed and cardiac samples were assayed by RT-PCR, immuno-blotting and -histology.

Findings: HFHS-diet induced type-2 diabetes (D) in WT and AS mice. VEGFa mRNAs were decreased in WT-D (–43%, $P<0.05$ vs. WT) while increased in AS-D mice (+236%, $P<0.01$ vs. WT-D). In WT-D hearts, the pro-apoptotic p38-MAPK was activated ($P<0.05$ vs. WT and AS-D) whereas Akt activity decreased. The AS-mice, that exhibited a cardiac upregulation of IGF1-R, showed an increase in Akt phosphorylation when diabetic ($P<0.05$ vs. WT and AS-D). Contrary to WT-D, AS-D hearts did not express inflammatory markers and exhibited a normal capillary density ($P<0.05$ vs. WT-D)

Conclusions: To our knowledge, this is the first study providing new insights into the mechanisms whereby aldosterone prevents diabetes-induced cardiac disorders.

0048

Deranged myofilament O'GlcNacylation and function in myocardium of obese patients

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The obesity-related cardiomyopathy is a poorly explained disease. There is no data available on myofilaments sensitivity to calcium and post-translationnal modification or isoform shifts of sarcomeric proteins that could be involved in the contractile impairment. We conducted a study on obese and non obese human atrial trabeculae of the right atrium collected in the surgery room during cardiopulmonary bypass. We studied the contractile force of the trabeculae, the sensitivity of the myofilaments to Ca2+. Western blots were performed in order to explore the post-translationnal modification of sarco-