ABSTRACT

Cardiovascular diseases account for the majority of deaths both worldwide and in the Czech Republic. Main factors contributing heart disease development, aside age and sex, are obesity, high blood pressure and high blood cholesterol and triglyceride levels. Spontaneously hypertensive rat (SHR) was developed and used for search of genetic determinants of these traits. This commonly used rat model develops hypertension, dyslipidemia, and insulin resistance naturally which is caused by aberrant Cd36 fatty acid translocase gene. Previous studies have shown that rescue of Cd36 performed in the transgenic SHR-Tg19 strain enhances cardiac beta-adrenergic system, slightly increases heart mass and leads to higher susceptibility to arrhythmias.

The present thesis had two main aims:

1) To investigate whether and how a transgenic rescue of Cd36 in SHR affects protein composition, mitochondrial function and activity of selected metabolic enzymes of the heart.

2) To study the expression and distribution of selected components of beta-adrenergic signaling system in lipid raft isolated form membranes using the TX-100 detergent.

We set to compare two commonly used proteomic approaches, 2D electrophoresis with MALDI-TOF mass spectrometry and label-free LC-MS. The results did not reveal any overlap between differently expressed proteins identified by these two methods. We also compared samples from both ventricles and found that both MALDI and LC-MS identified more changes in the RV of SHR-Tg19 than in the LV. These changes included several energy metabolism enzymes and cytoskeletal, structural and regulatory proteins. Changes in the LV included metabolic enzymes and, interestingly, translated products of pseudogenes similar to some OXPHOS enzymes, implicating their regulatory role.

Malate dehydrogenase, the enzyme that according to MALDI-TOF MS underwent a 6-fold downregulation in the LV of SHR-Tg19, had significantly lower activity in both cytoplasm and mitochondria samples of the LV from SHR-Tg19, as determined using an enzymatic assay. Activity of cytoplasmic hexokinase was also lower in the LV of SHR-Tg19. We also detected downregulated expression of the succinate dehydrogenase subunit SdhB (complex II) and 70 kDa peroxisomal membrane protein in the LV of SHR-Tg19. Although respirometric measurements did not reveal significant differences between the strains, our data demonstrated higher respiration rate of mitochondria isolated from the LV compared to RV.

We also adopted and elaborated a simplified method for lipid raft isolation from cardiac tissue. Feasibility of this methodology was tested by using a proteomic approach. The obtained results indicated that the method led to successful separation of typical raft and non-raft proteins. We used this method to analyze the distribution of several key components of beta-adrenergic signaling system in the LV of both rat strains. Expression of G protein beta subunit was lower in the raft fraction which could be linked to the enhanced cAMP signaling. Additionally, we found higher expression of connexin 43, which could be linked to increased arrhythmogenesis seen in SHR-Tg19, and higher expression of ERK1 and phosphorylation of RhoA, which may lead to an increase of heart mass observed in older SHR-Tg19. Connexin 43, ERK1 and RhoA are considered effectors of cAMP signaling network, thus showing its broad impact on heart function.