Abstract

Acute and chronic morphine administration can significantly reduce ischemia-reperfusion injury of the rat heart. However, the molecular mechanisms mediating the protective effect of morphine are not yet fully elucidated. Concurrently, there is a lack of information about the effects of the long-term action of morphine on heart tissue.

Therefore, in the first part of the project, we studied the effect of long-term administration of high doses of morphine (10 mg/kg/day, 10 days) on rat heart tissue. In the second part of the project, we investigated the effect of 1 mM morphine on viability and redox state of rat cardiomyoblast cell line H9c2 that was influenced by oxidative stress elicited by exposure to 300 μM *tert*-butyl hydroperoxide (*t*-BHP).

Our experiments have shown that long-term morphine administration affected neither the amount nor the affinity of myocardial β-adrenergic receptors (β-AR), but almost doubled the number of the dominant isoforms of myocardial adenylyl cyclase (AC) V/VI and led to supersensitization of AC. At the same time, proteomic analyses revealed that long-term morphine administration was associated with significant changes in the left ventricular proteome. In particular, there was an increase in the expression of heat shock proteins (HSP). Increased expression of HSP27 was concomitantly accompanied by increased phosphorylation of this protein. Whereas, after a 3-days drug abstinence phosphorylation of HSP27 further increased, after a 6-days abstinence its phosphorylation returned to the control level. Conversely, there were no alterations in the levels of proteins involved in the regulation of oxidative stress.

In the second part of our project, we have shown that 1 mM morphine can significantly protect the H9c2 cell line against oxidant injury induced by *t*-BHP. This protective effect of morphine was at least partially mediated by the simultaneous activation of the p38 MAPK and PI3K/GSK-3β signaling pathways. Morphine also increased the total antioxidant capacity of cells and reduced protein carbonylation, lipid peroxidation and production of reactive oxygen species. Preincubation with morphine reduced the negative impact of *t*-BHP on all the parameters of cellular redox state described above.

In summary, our experiments show that long-term administration of high doses of morphine to rats significantly affects myocardial AC signaling and leads to numerous changes in the cardiac tissue proteome. In vitro, morphine may have strong antioxidant effects and can considerably protect H9c2 cells against the oxidative stress.

Keywords: morphine, heart, proteomics, adenylyl cyclase, heat shock proteins, H9c2 cells, oxidative stress, PI3K, GSK-3β, p38 MAPK