2. Abstract

The aim of this dissertation was to evaluate the effect of low doses (0,1 mg/kg, 1 mg/kg, 1 mg/kg per day for 28 days + 7 days of abstinence) and high doses (10 mg/kg per day for 10 days) of morphine on transmembrane signaling mediated by G-protein coupled receptors (GPCRs) in the rat myocardium. Opioid receptors (OR) and mainly β-adrenergic receptors (β-AR) belong to the most important receptors of this receptor family.

δ-OR and κ-OR are the most numerous OR in the myocardium. Results of the present work indicated that there are no significant changes in the expression of these two receptor subtypes after any studied doses of morphine.

There are three subtypes of β-AR (β_1-AR, β_2-AR and β_3-AR) represent in the myocardial tissue. Here we studied the expression of β_1-AR and β_2-AR, because these two major subtypes of β-AR regulate through their signaling pathways functioning of the cardiovascular system. Our immunoblot analysis did not reveal any changes in the expression of β_1-AR, but the expression of β_2-AR was significantly decreased after treatment with morphine at the dose of 10 mg/kg for the 10 days. More sensitive saturation binding experiments with the nonselective β-antagonist [^{3}H]CGP 12177 indicated a significant increase in specific binding after treatment with morphine (1 mg/kg/day for 28 days) and, by contrast, there was a marked decrease after 7 days of drug withdrawal. β-AR are known to differ in their coupling to G-proteins. While β_1-AR exert their effect only through $G\alpha_s$ proteins, β_2-AR can additionally couple to pertussis toxin sensitive $G\alpha_i$ proteins. We did not observe any significant change in the expression of Gβ, $G\alpha_{i1/2}$, and $G\alpha_{q/11}$ proteins in samples after morphine exposure, but $G\alpha_{i3}$ subunit was increased.

We succeeded to isolate detergent-resistent microdomains (DRMs) by four different solubilizing agents (Triton X-100, CHAPS, sodium cholate, sodium carbonate). Tissue samples of control animals and those treated with morphine (10 mg/kg/day) showed different membrane localization of β_1-AR and β_2-AR. Whereas β_1-AR were present in the supernatant (solubilized proteins) as well as in the pellet (DRMs), β_2-AR were present almost exclusively in DRMs.

Keywords: β-adrenergic receptor, G-protein, GPCRs, morphine