ABSTRACT

In this Thesis, we studied properties of μ-, δ-, and κ-opioid receptors in lymphocytes isolated from rat spleen. This splenocytes were exposed to mitogen concanavalin A or opiate morphine and cultivated for 48 hours. Under physiological conditions, level of opioid receptors in immune cells is very low. Due to various factors such as presence of opioids, mitogens, long-term exposition to stress, expression of these receptors can be amplified. In this study we demonstrated, that concanavalin A causes up-regulation of μ-, δ- and κ-opioid receptors in lymphocytes isolated from rat spleen. In control cells no significant signal of μ- or δ-receptors was observed. In contrast, κ-opioid receptors were detected already in control cells. Concanavalin A stimulation caused a 2.4 - fold increase of these receptors.

In lymphocytes treated with morphine only μ-opioid receptors were up-regulated, whereas in control cells, there was no signal for these receptor type. δ-opioid receptors were not detected in control or morphine treated cells. κ-opioid receptors were determined in control and also in morphine affected lymphocytes but the amount of these receptors wasn´t changed by morphine.

Detection of μ-, δ- and κ-opioid receptors using Western blot technique in lymphocytes isolated from rat spleen, that were exposed to concanavalin A or morphine are published for the first time in this thesis.

Besides opioid receptors, three other proteins were established in rat spleen lymphocytes, which were exposed to concanavalin A. Actin, caveolin-1 and β-arrestin-1/2. These proteins participate in regulation and signaling of opioid receptors. Monomeric form of all these three proteins was down-regulated by concanavalin A.

Radioligand binding studies of opioid receptors has provided a negative result in both, control cells and in cells stimulated by concanavalin A. It´s possible, that amount of opioid receptors is below the detection limit of this method. Another interpretation could be that the newly synthesized receptors do not bind ligand and/or signaling pathways are not coupled to G-proteins.

Opioid receptors are mostly widespread in brain regions. Activation of opioid receptors in CNS by endogenous opioid peptides (enkephalins, endorphins) and exogenous opioids like morphine, codeine and heroin provide analgesic and euphoric effects. Repeated administration of these drugs causes negative side effects such as tolerance and dependence. Opioid receptors (μ-, δ-, κ-) were detected by Western blotting in rat brain

cortex. Animals were exposed to morphine or saline solution in case of control rats for 10 days. Amount of μ-opioid receptors wasn´t affected by morphine exposure. Small changes in level of minor forms of δ- and κ-opioid receptors were examined. Changes of these forms could be result of compensatory reactions that occur due to prolonged administration of morphine.

Key words: opioid receptors, structure-function relationships, morphine, lymphocytes