ABSTRACT

A huge effort has been put in determining the mechanism of the development of tolerance and dependence in context of clinical use of morphine for treatment of severe pain. Understanding of this mechanism would help to design new and more efficient pharmaceuticals. This diploma paper discus the opiate receptors with a special focus on long-term effect of chronic morphine treatment, which was determined using a radioligand binding assays with a non-selective antagonist [^{3}H]Diprenorphine. One of the goals of this work was to create and optimise a method for preparation of pure plasma membranes from rat cortex using percoll gradient. There were five groups, which differed in the length of morphine treatment: ten days (M-10), twenty-eight days (M-28), ten days with seven days of regression (RM-10 twenty-eight days with seven days of regression (RM-28) and a control group (K). The loss of total opioid receptor number was noticeable after ten days and grew slightly during continuous morphine treatment and kept lowering in the period of regression. The total loss was approximately 30% of the control binding. The equilibrium dissociation constant (K_d), thus the affinity of [^{3}H]Diprenorphine wasn't significantly different among the groups. Morphine acts through μ-opioid receptor, that's why there was a special effort to define the effect of long-term morphine treatment on this subtype using competitive binding study. A selective agonist DAMGO was used as a competitive ligand. The results shown, that after ten and twenty-eight days of morphine treatment there is an increase in percentage of μ-receptors present in the tissue. During the period of regression, this value regresses back to the control. The affinity of DAMGO to opioid receptors presented by K_i value remained the same among all groups. It was proved that the long-term morphine treatment lowers the total number of opioid binding in the rat cortex. After seven days of regression, there is still a significant loss of total receptor number. The percentage of receptors present in rat cortex is also changing.