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TIPA TOSKULKAO: GENE EXPRESSION OF OPIOID RECEPTORS AND G-PROTEIN SUBUNITS IN T LYMPHOCYTES OF HEROIN USERS. THESIS ADVISORS: PIYARAT GOVITRAPONG, Ph.D., NAIPHINICH KOTCHABHAKDI, Ph.D., VARAPORN AKKARAPATUMWONG, Ph.D., YUPIN SANVARINDA, Ph.D., SMITH VATANATUNYAKUM, M.D. 254 P. ISBN 974- 664-645-1.

Due to the AIDS epidemic, interest in studying the effects of drug abuse on the immune system has increased greatly. Our previous study indicated a depression in mitogen-stimulated T lymphocyte proliferation, both as an *in vitro* effect of morphine and in heroin addicts, including the modulation of surface markers observed on T cells. This immunosuppression is possibly mediated via the direct interaction of opiates with opioid receptors on T cells. In order to test this hypothesis, the levels of opioid receptor gene expression in the immune cells of heroin users were determined by using a semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) technique with specific pairs of primers to amplify mu- and delta-opioid receptor mRNAs. The level of both receptor mRNAs were expressed as a ratio to actin mRNA level. Both types of mRNAs on the T cells of heroin-addicted subjects were significantly decreased ($p<0.05$), with reversion to control levels in heroin-withdrawn subjects. mRNAs were also decreased in heroin-detoxification and methadone-maintenance subjects. Similarity, in an *in vitro* study, 10^{-4} M morphine treatment for 48 hr significantly down-regulated both mu- and delta-opioid receptor mRNA expressions in cultured T lymphocytes obtained from naïve subjects ($p<0.05$). This effect was prevented by the inclusion of 100 μM naloxone in the culture. The present data indicate that the gene expression of opioid receptors in human lymphocytes is down-regulated when cells are chronically exposed to opiates. To investigate the changes in the gene expression of specific subtypes of GTP-binding proteins (G-protein) in the T lymphocytes of heroin-addicted, heroin-withdrawn, heroin-detoxification, and methadone-maintenance subjects, a semi-quantitative RT-PCR assay was also used to amplify G_{α} and G_{β} mRNA subunits. Individual subunits were then identified by the restriction pattern of the RT-PCR products. The levels of G protein mRNA were expressed as a ratio to actin mRNA level. The $G_{\alpha i2}$ and $G_{\beta 1}$ mRNA levels were significantly increased from controls in heroin-addicted ($p<0.05, 0.01$), and heroin-withdrawn ($p<0.01$) subjects, while in the *in vitro* study, only the $G_{\beta 1}$ mRNA level was increased 12.35% from controls in the 10^{-4} M morphine-treated lymphocyte culture. The $G_{\alpha i2}$ mRNA level of heroin detoxification subjects was significantly decreased from controls ($p<0.05$). The mRNA level of $G_{\beta 2}$ was significantly decreased from controls in heroin-addicted ($p<0.05$), heroin-detoxification ($p<0.01$) and methadone-maintenance ($p<0.01$) subjects, but was significantly increased in the 10^{-4} M morphine-treated lymphocyte culture ($p<0.01$). The mRNA levels of $G_{\alpha i1}$ and $G_{\beta 3}$ were undetectable, both *in vivo* and *in vitro*, whereas, for $G_{\alpha o}$ and $G_{\alpha i3}$, only very weak signals could be detected. This work has shown that chronic heroin administration and methadone treatment cause specific changes of G-protein expression in T lymphocytes. The results obtained here can be used to further investigate the molecular changes that occur following the repetitive intake of drugs, as in cases of addiction. This is the first evidence showing alterations of lymphocyte opioid receptor and G-protein subunit gene expression in heroin users.