MORPHINE UPREGULATES KAPPA-OPIOID RECEPTORS OF HUMAN LYMPHOCYTES

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ABSTRACT

Opioids such as morphine are potent analgesic and addictive compounds. Chronic morphine use also induces immunomodulatory and immunosuppressive effects, as especially evident in HIV-infected patients. Morphine acts on the immune cells primarily through its binding to mu-opioid receptors on the plasma membrane. However, morphine modulation of immune functions still exists in mu-opioid receptor knockout mice, suggesting that in addition to the mu opioid receptors, morphine may also act by mechanisms mediated by either delta or kappa opioid receptors. To determine whether morphine activates kappa opioid receptors (KOR), a quantitative competitive RT-PCR procedure was utilized to quantify the KOR gene expression of morphine-treated cells. A segment of KOR transcript spanning the second extracellular loop, which has the reported dynorphin specificity, and the seventh transmembrane domain of the receptor was amplified from the total RNA of morphine-treated CEM x174 lymphocytes, along with a competitor molecule. The competitor was constructed by deleting a 33-nucleotide fragment from KOR. The results of the competitive RT/PCR indicated that CEMx174 cells expressed KOR mRNA constitutively, in the order of femto-grams. Treatment of 10 μM of morphine resulted in the up-regulation of KOR gene expression 24 hr post-treatment. The observed morphine effect could be reversed by treating the cells with either naloxone (a KOR-partially selective antagonist) or nor-Binaltorphimine (a KOR-selective antagonist).
INTRODUCTION

Immune cells have been shown to express brain-like kappa opioid receptors (KOR) both at transcriptional (Chuang et al., 1995) and translational level (Lawrence et al., 1995). However, the immunological functions of KOR remain largely unknown. Like mu or delta opioid receptors (Law and Loh, 1999), KOR belongs to the G-protein-coupled receptor family with seven transmembrane domains; ligand-binding studies indicate that KOR may selectively dimerize with delta but not with mu opioid receptors to form a new functional receptor (Jordan and Devi, 1999).

In a recent study we demonstrated that opioids, at micromolar concentrations, suppress the chemokine-mediated migration of both monkey neutrophils and monkey monocytes (Miyagi et al., 1999). Using various opioid receptor agonists and antagonists in the study we found that this inhibition of leukocyte migration by opioids is mediated by opioids binding to mu or kappa receptors; binding to delta opioid receptors was rarely observed (Miyagi et al., 1999). Morphine is a potent analgesic and addictive opioid; it also elicits various immunomodulatory and immunosuppressive effects on rhesus monkeys when used chronically, including suppression of T-cell proliferation response, IL-2 release (Chuang et al., 1993), inhibition of polymorphonuclear cell phagocytosis and chemotaxis (Liu et al., 1992), and alteration of the disease progression of simian immunodeficiency virus (SIV)-infected animals (Chuang et al., 1997). It is generally recognized that morphine induces its immunological actions through binding to its specific receptors, primarily of the mu type (Reisine and Pasternak, 1996). In mice lacking the mu opioid receptor gene, the morphine-induced immunosuppression was found to be abolished (Gaveriaux-Ruff et al., 1998). However, in another study, it was reported that several morphine-induced immune functions, including morphine reduction of splenic and thymic cell number and mitogen-induced proliferation, and morphine inhibition of IL-1 and IL-6 secretion by macrophages, are not affected in mu-opioid receptor-knockout mice, suggesting that morphine may act by a mechanism mediated by either delta or kappa opioid receptors (Roy et al., 1998). Resorting to an in vitro system, the present study was undertaken to determine whether morphine may indeed activate kappa opioid receptors of immune cells.

MATERIALS AND METHODS

Cell Culture

The CEM x 174 cell line, a hybrid of human B cell line 721. 174 and human T cell line CEM (Salter et al., 1985), was used as a model system to determine the effect of morphine on KOR gene expression of immune cells.

Treatment with morphine and/or antagonists

Morphine sulfate, naloxone (a KOR receptors-partially selective antagonist, Reisine and Pasternak, 1996), nor-Binaltrophimine (nor-BNI, a KOR receptors-selective antagonist, Reisine and Pasternak, 1996), and naltrindole (NTI, a delta opioid receptors-selective antagonist, Reisine