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κ opioid receptors in human microglia downregulate human immunodeficiency virus 1 expression

(U50,488/U69,593)

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ABSTRACT **Microglial cells, the resident macrophages of the brain, play an important role in the neuropathogenesis of human immunodeficiency virus type 1 (HIV-1), and recent studies suggest that opioid peptides regulate the function of macrophages from somatic tissues. We report herein the presence of κ opioid receptors (KORs) in human fetal microglia and inhibition of HIV-1 expression in acutely infected microglial cell cultures treated with KOR ligands. Using reverse transcriptase–polymerase chain reaction and sequencing analyses, we found that mRNA for the KOR was constitutively expressed in microglia and determined that the nucleotide sequence of the open reading frame was identical to that of the human brain KOR gene. The expression of KOR in microglial cells was confirmed by membrane binding of [^{3}H]U69,593, a κ-selective ligand, and by indirect immunofluorescence. Treatment of microglial cell cultures with U50,488 or U69,593 resulted in a dose-dependent inhibition of expression of the monocytotropic HIV-1 SF162 strain. This antiviral effect of the κ ligands was blocked by the specific KOR antagonist, nor-binaltrophimine. These findings suggest that κ opioid agonists have immunomodulatory activity in the brain, and that these compounds could have potential in the treatment of HIV-1-associated encephalopathy.**

Activation of the three major types of opioid receptors (i.e., μ, δ, and κ) has been shown to affect the endocrine, cardiovascular, respiratory, gastrointestinal, and nervous systems (1, 2). Considerable evidence has been amassed over the past decade or more which indicates that endogenous opioid peptides also affect the immune system (3). In recent years, increased attention has been paid to the immunosuppressive effects of κ opioid ligands on macrophages (4–6) as well as on lymphocytes (7–10). These studies have suggested the existence of κ opioid receptors (KORs) on these immune cells. Pharmacological studies using the KOR-specific ligand [^{3}H]U69,593 have now confirmed a κ opioid binding site on the macrophage cell line P388d$_1$ with a K_D value of 17 nM (4) and on the mouse R1.1 thymoma cell line with a K_D value of 0.2 nM (8). Using a high-affinity fluorescein-conjugated κ opioid ligand and an indirect immunofluorescence technique, Lawrence *et al.* (11) have provided evidence for the presence of KOR on murine thymocytes. Also, Chuang *et al.* (12) have reported the partial KOR amino acid sequences deduced from cDNA sequences of human and monkey CD4 lymphocytes. The expression of the KOR and the biologic function of the KOR on mononuclear phagocytes, however, have not been defined.

Widespread distribution of the three types of opioid receptors has been demonstrated within the central nervous system (CNS) (13). Recently, the KOR cDNA has been cloned from a human brain cDNA library (14), and immunocytochemical staining revealed the presence of the neuronal KOR in postsynaptic sites (15, 16). Over 80% of the cells in the cerebral cortex, however, are glial cells, and there is suggestive evidence for KOR in rodent astrocytes (17–19), the predominant cell type within the brain. Microglia, the resident macrophages of the brain, comprise about 5% of the cells in the cerebral cortex. In the present study, we hypothesized that microglia possess KOR and that stimulation of microglial cell KOR would have functional consequences. Because brain macrophages are the principal cell type in which human immunodeficiency virus type 1 (HIV-1) replicates within the CNS (20, 21), we specifically examined the effect of κ-selective ligands on HIV-1 expression in acutely infected microglial cell cultures.

MATERIALS AND METHODS

Reagents. The monocytotropic SF162 strain of HIV-1 was kindly provided by the National Institutes of Health AIDS Research and Reference Reagent Program (National Institute of Allergy and Infectious Diseases, Rockville, MD). U50,488 and U69,593 were gifts of The Upjohn Company (Kalamazoo, MI), and (+)U50,488 and (−)U50,488 stereoisomers were kindly provided by Kenner Rice (National Institutes of Health). Dynorphin A$_{1-13}$ and $_{1-17}$ were purchased from Peninsula Laboratories. The κ-selective antagonist nor-binaltrophimine (nor-BNI) was kindly provided by P. S. Portoghese (University of Minnesota, Minneapolis). The fluorescein-isothiocynate-coupled arylacetamide κ opioid agonist 2-(3, 4-dichlorophenyl)-*N*-methyl-*N*-[1-(3-aminophenyl)2-(1-pyrrolidinyl)ethyl] acetamide (FITC-AA) was prepared as described (11). Other reagents were purchased from the indicated sources: dynorphin$_{1-13}$ (Peninsula Laboratories); [^{3}H]U69,593 (54 Ci/mmol; 1 Ci = 37 GBq) (Amersham); biotinylated rabbit anti-fluorescein IgG (Molecular Probes); antibodies to microglial cell CD68 antigen and astrocyte glial fibrillary acid protein (Dako); oligo(dT)$_{12-18}$ primer (Clontech); *Taq* DNA polymerase, spermidine, avian myeloblastosis virus reverse transcriptase (RT), and PolyATtract mRNA Isolation System III (Promega); dexoynucleotide triphosphate (dNTP) mixture containing dATP, dTTP, dGTP and dCTP (Boehringer Mannheim); RNase inhibitor (Pharmacia); fetal bovine serum (HyClone Laboratories); extravidin-R-phycoerythrin, Dulbecco's modified Eagle's medium, Hepes-

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Abbreviations: Ag, antigen; CNS, central nervous system; FITC-AA, fluorescein-isothiocynate-coupled arylacetamide; HIV, human immunodeficiency virus; KOR, κ opioid receptor; nor-BNI, nor-binaltrophimine; ORF, open reading frame; RT-PCR, reverse transcriptase–PCR.

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buffered balanced salt solution, penicillin (100 units/ml), streptomycin, and all other culture reagents (Sigma).

Microglial Cell Cultures. Human fetal brain tissue was obtained from 16- to 22-week old aborted fetuses under a protocol approved by the Human Subjects Research Committee at our institution. The procedure for isolating microglial cells has been described (22). Briefly, brain tissues were dissociated following a 30-min trypsinization (0.25%) and plated in 75 cm^2 Falcon culture flasks in medium containing 10% heat-inactivated fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). The medium was replenished 1 and 4 days after plating in medium containing 10% fetal bovine serum only. On day 12 of culture, plates were gently shaken and harvested cells were filtered through a 70-μm nylon mesh. These cells were then plated onto wells for experimentation. Purified microglia were composed of a cell population of which >99% stained with anti-CD68 antibody (a human macrophage marker) and <1% stained with anti-glial fibrillary acid protein antibody (an astrocyte marker).

Expression of KOR mRNA and Sequencing Analysis. To determine whether microglial cells constitutively express KOR sequences, a RT–PCR technique was used to detect expression of the KOR gene in untreated microglial cell cultures. Total RNA was isolated as described (23). Initially, one pair of oligonucleotide primers, hkappa$^+$ and hkappa$^-$ (Fig. 1), was selected based on the sequences from GenBank accession number L37362 (14). These primers amplify a 388-bp cDNA fragment corresponding to the region from the putative second extracellular loop to the seventh transmembrane domain of both the human brain and placenta KOR cDNA (14, 24). The extracellular loop domain was chosen since it was reported that this region contains the sequence contributing to KOR's selectivity in binding the dynorphin ligand (25). For sequence analysis of the entire KOR open reading frame (ORF), four additional sets of primers (k1, k3, k6, and k7) were also selected (see Fig. 1).

Reverse transcription of 1 μg total RNA from resting microglial cell cultures was performed by using the oligo(dT)$_{12-18}$ primer and avian myeloblastosis virus RT. Amplification of the KOR cDNA was performed in a final reaction volume of 50 μl consisting of 5 μl of 10× PCR buffer (500 mM KCl/100 mM Tris·HCl, pH 9.0/1% Triton X-100), 3 μl of 25 mM $MgCl_2$, 1 μl of dNTP mixture (10 mM of each dATP, dTTP, dGTP, and dCTP), 1 μl of each (sense and anti-sense) primer (from a 25 μM stock), 2 μl of cDNA, and 2 units of *Taq* DNA polymerase. The mixture was subjected to 35 amplification cycles with each cycle as follows: 94°C for 45 sec, 65°C for 45 sec, and 72°C for 90 sec. The amplified cDNA fragment was analyzed and visualized by 1.5% agarose gel electrophoresis, cloned into Bluescript plasmid, and sequenced using a Sequenase version 2.0 sequencing kit (United States Biochemical). After sequence analysis confirmed that the PCR product was indeed KOR cDNA, the entire ORF was amplified in segments and sequenced. To facilitate sequencing of the ORF, total RNA was further purified to poly(A)$^+$ mRNA using a PolyATtract mRNA Isolation kit. cDNA was synthesized as described above, using ≈40 ng mRNA per reaction. The cDNA was amplified by using the remaining primer pairs, and PCR products were sequenced directly by an automated fluorescent method (Applied Biosystems; DNA Sequencing Facility, Iowa State University, Ames).

Competitive Binding Studies with [^{3}H]U69,593. To test microglial cell membranes for KOR, binding studies were performed as described (8). Membranes (100 μg of protein each treatment tube) from human fetal microglial cells (10^7 cells) were incubated with 1 nM [^{3}H]U69,593 in the absence or presence of 10 μM nor-BNI, in a final volume of 1 ml of 50 mM Tris·HCl (pH 7.5). After incubation for 60 min at 25°C, samples were filtered through glass fiber filters that were presoaked in 0.25% polyethylenimine. The filters were washed with ice-cold

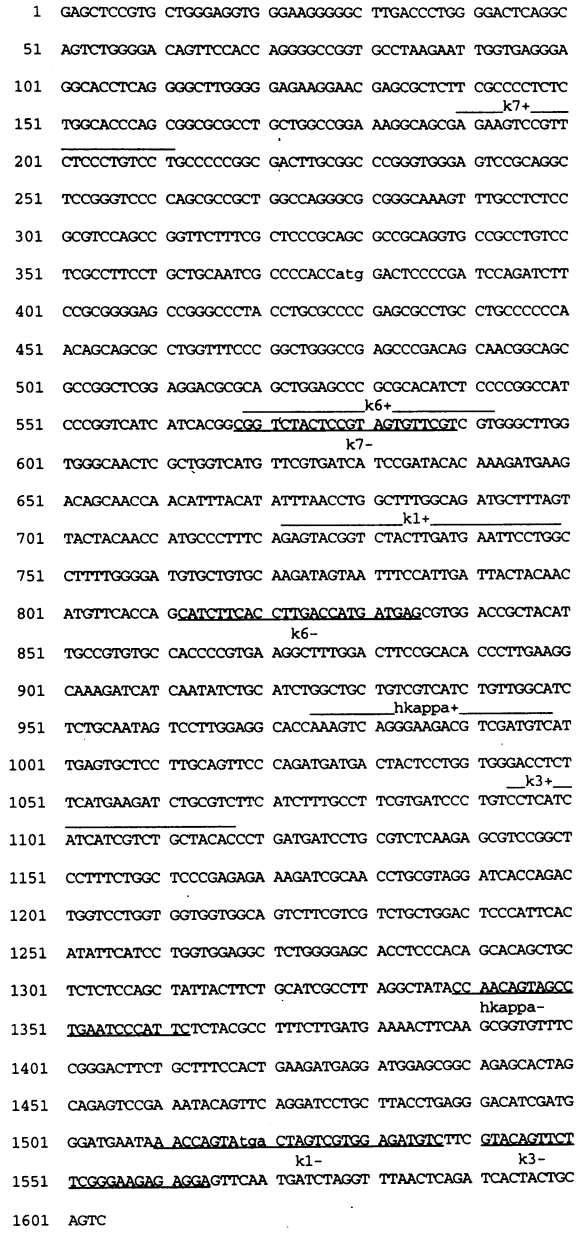

FIG. 1. cDNA sequence of KOR from human brain (GenBank accession no. L37362), showing location of primers used for PCR amplification of human microglial cell cDNA. Primer sequences are overlined indicating sense (+) strands or underlined indicating anti-sense (−) strands.

Tris·HCl (pH 7.5), then counted in 2 ml of Ecoscint A scintillation cocktail (Research Biochemicals, Natick, MA). Selection of 1 nM [^{3}H]U69,593 in the present study was based on a previous study with the R1.1 cell line (8) and a preliminary study indicating that the K_D value of [^{3}H]U69,593 binding to human glial cell membranes was 0.4 nM (unpublished data).

Indirect Immunofluorescence with Phycoerythrin. For all fluorescence studies, the buffer used was a Hepes-buffered balanced salt solution, which consisted of 15 mM Hepes, 3.4 mM K_2HPO_4, 0.6 mM KH_2PO_4, 150 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 1% (wt/vol) bovine serum albumin (pH 7.4) as described (11). Unfixed cells were washed twice by centrifugation at 200 × g for 10 min at 4°C, followed by resuspension in medium. In a final volume of 100 μl medium, 2 × 10^5 cells per sample were incubated with 30 μM FITC-AA for 30 min at 25°C for optimal staining. This volume included 10 μl of biotin-conjugated anti-fluorescein IgG,

except in FITC-AA only controls as described below. The high-affinity, κ-selective antagonist nor-BNI at a final concentration of 500 μM was included to measure nonspecific fluorescence. Samples were incubated for 30 min on ice, diluted with 1 ml of medium, followed by centrifugation at 400 × *g* for 3 min at 4°C. After aspirating the supernatants, cells were washed twice, then resuspended in 40 μl of medium and 10 μl of extravidin-conjugated R-phycoerythrin. This incubation took place for 15 min on ice, then the cells were washed twice before resuspension in a final volume of 500 μl medium. Samples were viewed with an Olympus BH-2 fluorescence microscope, using a NMG filter cube with independent filters to visualize fluorescein or phycoerythrin fluorescence, as described (11). The objective used was a D-Plan Apo ×20 with an aperture of 0.70.

In each sample, 10,000–15,000 cells were analyzed using a Coulter XL100. The fluorescein was measured on a logarithmic scale, with band pass filters of 530 nm for fluorescein and 585 nm for phycoerythrin. Median values of fluorescence intensity distributions were used to compare the fluorescence among samples. This assumes that only a single population is labeled, because the resulting histograms were bell-shaped, consistent with a single population of cellular staining. A small (<10%) cell population that contained high autofluorescence was excluded. Cells labeled with only FITC-AA were used as negative controls to ensure that fluorescein did not contribute to the phycoerythrin signal as measured by the flow cytometer. This was accomplished by setting the compensation such that when phycoerythrin fluorescence was measured, the intensity of cells labeled with only FITC-AA was identical to that of the autofluorescence controls. Background phycoerythrin controls were also included, where FITC-AA was omitted but cells were incubated with biotin-conjugated anti-fluorescein IgG and extravidin-conjugated R-phycoerythrin. This control served to reestablish a baseline of R-phycoerythrin emission, taking into consideration the nonspecific staining of both the anti-fluorescein IgG and the extravidin-R-phycoerythrin.

Effects of KOR Stimulation on HIV-1 Expression in Acutely Infected Microglial Cell Cultures. To evaluate the effect of KOR activation on HIV-1 expression in microglial cell cultures, we modified a previously described technique by using mixed glial and neuronal cell cultures infected with the monocytotropic strain HIV-1 SF162 (26). In brief, microglial cells (2×10^5/well) were first treated with indicated concentrations of U50,488 for 24 h followed by infection with the SF162 strain at a multiplicity of infection of 0.02 for 24 h according to a previously described protocol (27). After washing three times, cell cultures were incubated for 7, 14, or 21 days, and supernatants were harvested for quantifying HIV-1 p24 antigen (Ag) as a reflection of HIV-1 expression, as described (23).

Next, we evaluated the effects of several κ opioid ligands (i.e., U50,488, U69,593, dynorphin A_{1-13}, and dynorphin A_{1-17}) on HIV-1 expression in acutely infected microglial cell cultures. Cell cultures were first treated with varying concentrations (ranging from 0.1 fM to 1 μM) of these κ-selective ligands for 24 h prior to infection with HIV-1 for an additional 24 h. Supernatants were harvested on day 7 of culture and assayed for p24 Ag. To test the stereospecificity of U50,488, a dose-response curve ranging from 10 fM to 1 μM of (−) or (+) isomers was carried out. The specificity of KOR activation was confirmed by adding a 10-fold higher concentration of nor-BNI 30 min before treatment with κ-selective ligands.

HIV-1 Ag levels were measured by using an enzyme-linked immunosorbent assay that detects mainly HIV-1 p24 Ag (Abbott Laboratories), as described (23). A standard curve derived from known amounts of p24 Ag was used to quantify the Ag levels in culture supernatants.

Statistical Analysis. Where appropriate, data were expressed as mean ± SEM. To compare means of two groups, Student's *t* test was used.

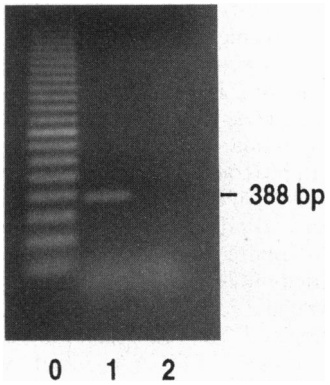

FIG. 2. Constitutive expression of KOR mRNA. Total RNA was harvested from resting microglial cell cultures. RT–PCR was performed to evaluate constitutive expression of KOR mRNA. RT–PCR products were loaded onto a 1.5% agarose gel. Lanes: 0, molecular weight marker; 1, RT–PCR product; 2, negative control (RT–PCR in the absence of RT).

RESULTS

Expression of KOR mRNA and Sequencing Analysis. Using RT–PCR analysis with a primer set for amplification of hkappa cDNA, we found that human microglia constitutively express KOR mRNA. This was not found in the negative control group where RT was omitted, thereby excluding the possibility of an artifact due to genomic DNA contamination (Fig. 2). DNA sequence analysis of overlapping RT–PCR products derived from the five primer sets shown in Fig. 1 revealed 100% identity between microglial cell cDNA and the human brain KOR gene (14). Comparison of the deduced amino acid sequence from microglial KOR cDNA to that of human placenta (24) and CD4 lymphocytes (12) also showed nearly 100% homology.

Competitive Binding Studies with [^{3}H]U69,593. In addition to molecular evidence of constitutive expression of microglial cell KOR, we also found that the KOR-selective ligand [^{3}H]U69,593 (1 nM) bound to a microglial cell membrane preparation (35.22 ± 1.43 fmol/mg protein). The binding of the radiolabeled ligand was blocked by 63.5% ($P < 0.01$) in the presence of nor-BNI (12.85 ± 1.75 fmol/mg protein) (Fig. 3). The K_D value for [^{3}H]U69,593 binding to human microglial cell membranes was not carried out because of the technical difficulty in obtaining a sufficient number of microglial cells for such a study.

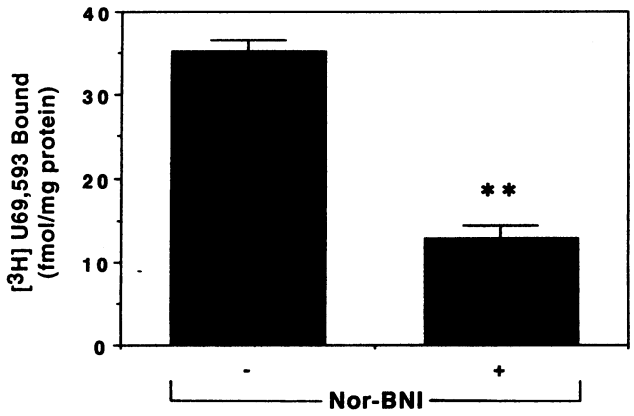

FIG. 3. Specific binding of [^{3}H]U69,593 to human microglial cell membranes. Binding of [^{3}H]U69,593 (1 nM) was performed by using human microglial cell membranes (100 μg of protein/sample) in the absence or presence of 10 μM nor-BNI. Data are mean ± SEM of duplicates. **, $P < 0.01$ versus group without nor-BNI.

Indirect Immunofluorescence. Visualization of binding of a κ-selective ligand to microglial cells at a cellular level was accomplished by using an indirect immunofluorescence microscopy technique and FITC-AA, a κ-selective ligand. This technique revealed clear binding of FITC-AA to microglial cells (Fig. 4*B*) as compared with control cells (Fig. 4*A*) after amplification with phycoerythrin-conjugated antibody against FITC. More than 90% of the FITC-AA-treated microglial cells stained positively. Binding of FITC-AA was completely blocked by pretreatment for 30 min with nor-BNI (Fig. 4*C*), suggesting specificity for the KOR. To confirm the binding of FITC-AA to microglial cells, fluorescence-activated cell sorter analysis was performed. This analysis revealed that 98% of the microglial cells bound FITC-AA, which was completely blocked by nor-BNI (Fig. 5). The fluorescence intensity of FITC-AA binding (median 42.2 arbitrary units versus 2.93 arbitrary units in antibody control group) was totally blocked by pretreatment of microglial cells with nor-BNI (2.3 arbitrary units). The median intensity of the autofluorescence control (FITC-AA only without antibody) was 5.6 arbitrary units.

Effect of KOR Activation on HIV-1 Expression in Acutely Infected Microglial Cell Cultures. To evaluate the effect of stimulation of microglial cell KOR on HIV-1 expression, we first developed an *in vitro* model of microglial cell HIV-1 infection by using the monocytotropic SF162 strain. Expression of HIV-1 was detected 7 days after infection of human microglial cells, peaking by 21 days (Fig. 6). In 10 separate experiments, the control levels of HIV-1 p24 Ag obtained from supernatants of 7-day cultures were 489 ± 96 pg/ml, ranging from 124 to 897 pg/ml. Treatment of microglial cell cultures with either U50,488 or U69,593 resulted in a dose-dependent, bell-shaped inhibition of HIV-1 expression (Fig. 7). Maximal inhibition was observed when microglial cell cultures were treated with 1 pM U50,488 (≈47% suppression) and 10 fM U69,593 (≈52% inhibition), respectively. On the other hand, the endogenous KOR ligand, dynorphin A_{1-13}, induced minimal inhibition of HIV-1 expression (maximal inhibition was 16.9% at a concentration of 100 pM). Dynorphin A_{1-17} did not affect HIV-1 expression in this model system (data not shown). This finding suggests that activation of the KOR by the stable synthetic KOR ligands is associated with a more potent inhibition of HIV-1 expression. In a separate experiment, the inactive enantiomer (+) U50,488 (1 pM) inhibited HIV-1 expression by only 12% ($P > 0.05$), while (−) U50,488 suppressed HIV-1 expression by 43% ($P < 0.01$), suggesting the inhibitory effect of this KOR ligand is stereospecific. Pretreatment of microglial cell cultures with 10 pM nor-BNI

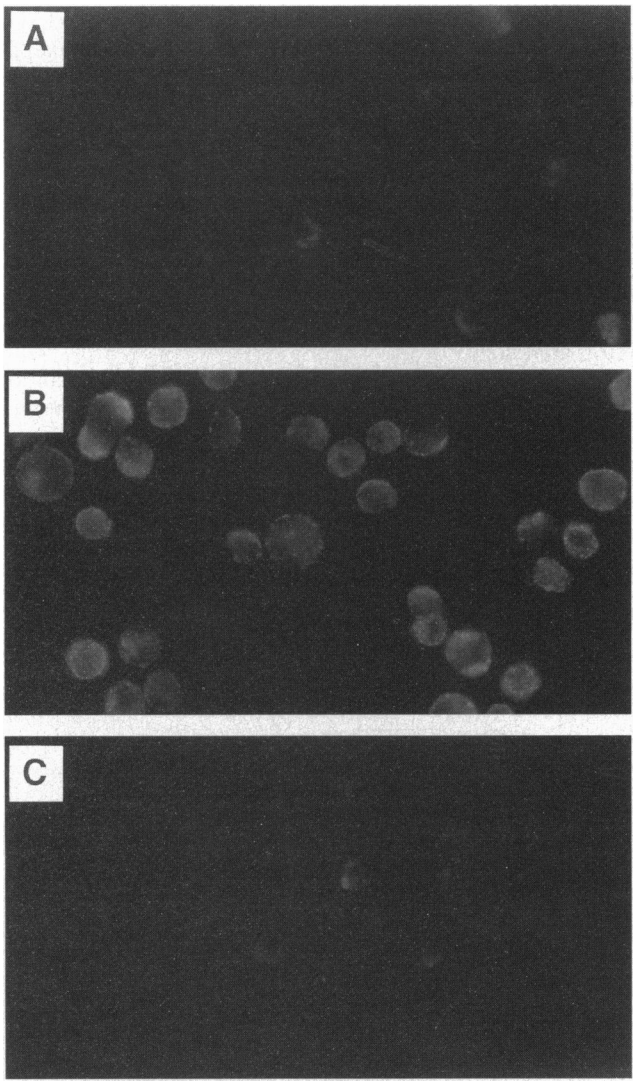

FIG. 4. Microscopic analysis of phycoerythrin amplification of FITC-AA labeling of the KOR. Microglial cells were incubated with Hepes-balanced salt solution buffer (*A*), 30 μM FITC-AA (*B*), or 30 μM FITC-AA plus 500 μM nor-BNI (*C*), followed by incubations with biotinylated anti-fluorescein IgG and extravidin-coupled R-phycoerythrin. (×400)

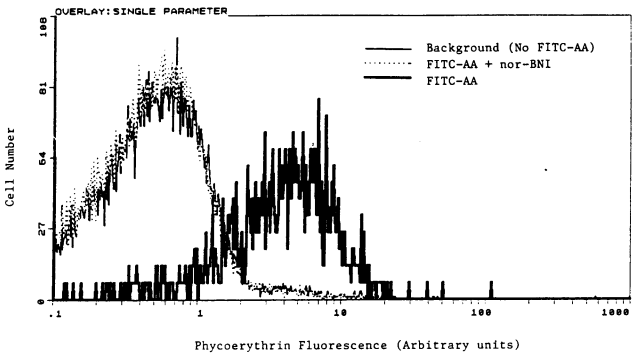

FIG. 5. Flow cytometric analysis of phycoerythrin amplification of FITC-AA labeling of the KOR. Microglial cells were incubated with 30 μM FITC-AA in the absence or presence of 500 μM nor-BNI or without FITC-AA (only biotinylated anti-fluorescein IgG and extravidin-coupled R-phycoerythrin) as a background control. Data are representative of three separate experiments.

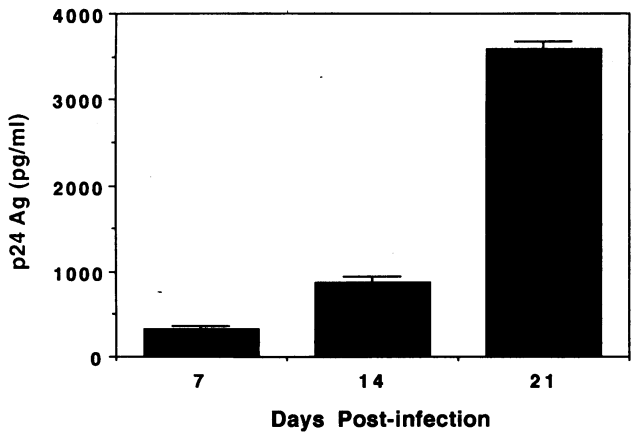

FIG. 6. HIV-1 expression in acutely infected microglial cell cultures. HIV-1 SF162 was added to microglial cell cultures for 24 h. After washing, cell cultures were incubated for indicated time periods and supernatants were harvested for assaying p24 Ag. Data are mean ± SEM of duplicate values and are representative of three experiments.

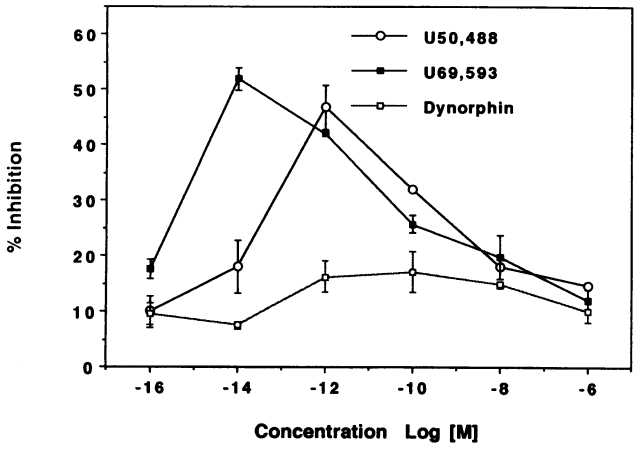

FIG. 7. Effects of κ-selective ligands on HIV-1 expression. Microglial cells were treated with indicated concentrations of U50,488, U69,593, or dynorphin A_{1-13} for 24 h before incubation with HIV-1 for 24 h. After washing, cultures were incubated for 7 days at which time supernatants were harvested for measurement of p24 Ag levels. Data are expressed as % inhibition relative to HIV-1 p24 Ag levels in supernatants of control cells lacking opioids (652 ± 35 pg p24 Ag/ml). Values are mean ± SEM of duplicates and are representative of three experiments.

markedly attenuated ($P < 0.01$) the inhibitory effect of U50,488 and U69,593 on HIV-1 expression (Fig. 8).

DISCUSSION

Our results have revealed that human microglial cells constitutively express KOR, as demonstrated by molecular (RT–PCR and sequence analysis), pharmacological (radioligand binding), and immunological (indirect immunofluorescence) techniques. Recently, the deduced KOR amino acid sequence also has been reported in human and monkey T lymphocytes (12), and it appears that the sequence of KOR in both types of immune cells is similar or identical to that described in human brain tissue (14). In the present study, we have provided functional evidence of the KOR showing inhibition of HIV-1 expression in acutely infected microglial cells by KOR ligands.

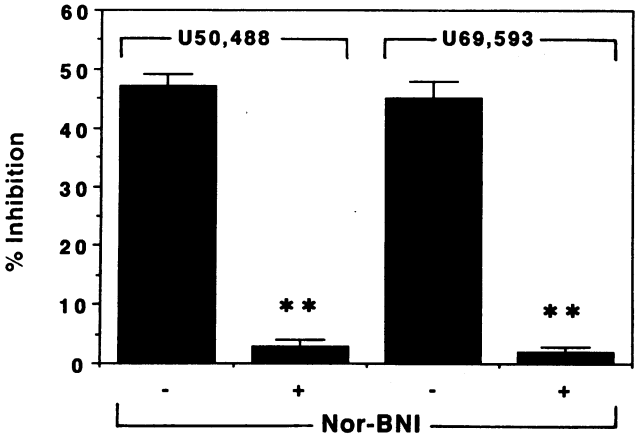

FIG. 8. Nor-BNI blockade of κ agonist-mediated-inhibition of HIV-1 expression. Microglial cells were treated with medium (−) or 10 pM nor-BNI (+) for 30 min before incubation with 1 pM U50,499 or 10 fM U69,593 for 24 h followed by infection with HIV-1. After 7 days of incubation, supernatants were harvested for assaying p24 Ag levels. Data are expressed as % inhibition. Values are mean ± SEM of duplicates and are representative of three experiments. **, $P < 0.01$ versus group without nor-BNI.

Microglia are derived from blood monocytes arising from the bone marrow early in embryonic development (28). These resident macrophages of the brain are the ontogenetic and functional equivalent of macrophages in somatic tissues (29). Histopathological evidence suggests that microglia play an important role in the neuropathogenesis of HIV-1 infection within the CNS (30–33). Permissive HIV-1 infection of the CNS occurs only in microglia, although astrocytes are also involved in HIV-1-related neuropathogenesis (34, 35). While antiviral drugs such as RT (26, 36) and protease (26) inhibitors have been shown to suppress HIV-1 expression in brain cell cultures, the finding that U50,488 and U69,593 inhibit viral expression suggests that targeting relevant microglial cell receptors, such as KOR, may be an effective strategy to inhibit HIV-1 by an indirect mechanism.

Dynorphin, the endogenous KOR ligand, is distributed throughout the CNS (37). Although dynorphin possesses higher affinity for KOR, this opioid peptide also binds to μ opioid receptors (MOR) (selective for morphine-like ligands) and δ receptors (enkephalin selective) as well as to nonopioid receptor sites (38). The demonstration of 62% homology between human brain KOR and MOR suggests a potential for functional overlap. In addition to involvement in neurotransmitter release (39, 40), the biologic activities associated with KOR include suppression of opiate withdrawal and tolerance (41), stress-induced behavior (42), food intake (43), memory (44), and glutamate receptor-mediated neurotoxicity (45). The finding that κ-selective ligands inhibit HIV-1 expression in microglial cell cultures supports the concept that the KOR also has an immunomodulatory role in the CNS. That the KOR ligands U50,488 and U69,593 were more potent than dynorphin in suppressing HIV-1 expression is likely due to resistance of these synthetic compounds to endopeptidases to which dynorphin is highly susceptible (46). In this regard, U50,488 has been shown to have superior neuroprotective activity against ischemic injury when compared with dynorphin (47).

Previous studies in our laboratory have indicated that both morphine and the KOR ligands (i.e., dynorphin and U50,488) potentiate expression of HIV-1 in chronically infected promonocytes (U1 cells) when these cells were cocultured with human fetal mixed glial/neuronal cells (23, 48). The mechanism of the indirect proviral effect of these opioids appears to involve an opioid receptor and an enhanced production of cytokines known to upregulate the expression of HIV-1 in U1 cells (23, 48). The mechanism underlying the antiviral effect of KOR ligands in acutely infected microglial cell cultures in the present study is unknown. The discrepancy between an antiviral effect of U50,488 versus a proviral effect in previous studies of mixed brain/U1 cell cocultures could be explained by several factors: the type of culture (enriched microglial cells versus mixed brain/U1 cocultures), the type of HIV-1 infection (acutely versus chronically infected cells), viral strain (HIV-1 SF162 versus AD-87), and the cell source (brain microglia versus somatic promonocytes). A question remaining to be addressed is which infection model has more relevance to human HIV-1 encephalopathy and AIDS dementia. The clinical observation that RT inhibitors are beneficial in the treatment of HIV-1-induced encephalopathy and yet have little or no effect on limiting HIV-1 expression in chronically infected promonocytes (26, 49, 50) suggests that acutely infected microglial cells are important in the neuropathogenesis of HIV-1. If so, the findings in the present study that KOR agonists inhibited HIV-1 expression may have therapeutic implications.

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