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RAPID COMMUNICATION

δ-Opioid Suppression of Human Immunodeficiency Virus-1 Expression in T Cells (Jurkat)

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ABSTRACT. δ-opioid receptor (DOR) transcripts and binding sites are expressed by lymphocytes and lymphoid cell lines from several species. Direct modulation of lymphocyte function through DORs affects T cell proliferation, interleukin-2 production, chemotaxis, and intracellular signaling. Moreover, in human DOR-transfected T cells (DOR-Ju.1), δ-opioids have been shown previously to mobilize intracellular calcium rapidly, to inhibit forskolin-stimulated cyclic AMP production, and to activate the mitogen-activated protein kinases ERKs 1 and 2. These observations led us to consider whether δ agonists modify T cell functions, thus affecting the expression of human immunodeficiency virus-1 (HIV-1) by $CD4^+$ T cells. To test this hypothesis, DOR-Ju.1 cells, derived from Jurkat cells stably transfected with a cDNA encoding the neuronal DOR, were stimulated with deltorphin or benzamide, 4-[[2,5-dimethyl-4-(2-propenyl)-1-piperazinyl](3-methoxyphenyl)methyl]*N*-,[2*S*[(*S**),2α,5β]]-(9Cl) (SNC-80) prior to the addition of HIV-1. Both deltorphin and SNC-80 concentration-dependently inhibited the production of p24 antigen, an index of HIV-1 expression. Inhibition was maximal with 10^{-13}–10^{-9} M SNC-80 (>60% reduction) or 10^{-15}–10^{-11} M deltorphin (>50% reduction). At higher concentrations, less inhibition of p24 antigen production was found. Naltrindole (NTI, 10^{-11} M), a selective DOR antagonist, abolished the inhibitory effects of 10^{-9} M SNC-80, whereas 10^{-13} M NTI partially reversed the effect of SNC-80. Thus, activation of DORs expressed by $CD4^+$ T cells significantly ($P < 0.05$) reduced the expression of HIV-1 by these cells. These findings suggest that opioid immunomodulation directed at host T cells may be adjunctive to standard antiviral approaches to HIV-1 infection. BIOCHEM PHARMACOL **56**;3:289–292, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. SNC-80; deltorphin; δ opioid peptides; naltrindole; HIV-1; immunomodulation

δ-opioid agonists affect a multitude of immune functions involving lymphocytes in the thymus, spleen, and peripheral circulation. Some of these actions appear to mimic the effects of the endogenous opioid peptides produced by lymphocytes. For example, $CD4^+$ murine thymocytes express preproenkephalin A mRNA, and enkephalin peptides have been identified in splenic extracts [1, 2]. Acting through DORs§, endogenous enkephalins appear to modulate thymocyte proliferation in response to con A [3]. Pharmacological studies have also shown that DOR agonists modulate proliferation and interleukin-2 production by highly purified $CD4^+$ and $CD8^+$ T cells stimulated through the T cell antigen receptor complex [4]. Additionally, DOR ligands have been shown to affect lymphocyte intracellular signaling. Thus, methionine enkephalin exerted biphasic effects on cAMP levels in human peripheral blood lymphocytes, and β-endorphin (antagonized by DOR selective naltrindole) or DADLE enhanced the con A-induced mobilization of intracellular free calcium by murine splenic T cells [5, 6].

DOR transcripts have been found recently in mononuclear cells from several species. To detect DOR mRNA in simian peripheral blood mononuclear cells, human peripheral blood lymphocytes, and murine splenocytes, several laboratories have used reverse transcription–polymerase chain reaction techniques‖ [7–10]. DOR transcripts were identified in freshly obtained simian mononuclear cells and murine splenocytes‖ [7, 10], and expression in murine splenic T cells was enhanced by cell culture (unstimulated), con A, and cross-linking the T cell antigen receptor with anti-CD_3-ε‖ [9, 10]. Apparently, mitogenic stimulation with phytohemagglutinin is required to detect DOR transcripts in human peripheral blood lymphocytes [8]. Thus,

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§ *Abbreviations:* cAMP, cyclic AMP; con A, concanavalin A; DADLE, [D-Ala^2, D-Leu^5]-enkephalin; DOR, δ-opioid receptors; and HIV-1, human immunodeficiency virus-1.

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low levels of DOR mRNA are present in lymphocytes in the systemic circulation of several species, and substantial induction takes place upon activation.

Complementary studies that demonstrate binding to leukocyte DORs are highly limited. One study reported that [^{3}H]deltorphin binds to a single high affinity site (K_d = 0.45 nM) on membranes from human peripheral blood polymorphonuclear leukocytes [11]. Additionally, covalent cross-linking with a δ-selective ligand, *cis*(+)-3-methylfentanylisothiocyanate (SUPERFIT), suggested the presence of DORs on both murine T cell-enriched splenocytes and human peripheral blood lymphocytes [12].

Previous studies have shown that morphine enhances HIV-1 propagation through mu-like opioid receptors on acutely infected peripheral blood mononuclear cells [13]. The strong functional evidence for DORs on T cells, along with the presence of DOR transcripts, and the central role of $CD4^+$ molecules in the uptake of HIV-1 by human T cells led us to hypothesize that DOR agonists may modulate the expression of HIV-1 in $CD4^+$ T cells. To test the concept that DORs modulate HIV-1 expression, a human T cell line derived from Jurkat cells and stably transfected with a DOR cDNA was used [14]. DOR ligands affect several intracellular signal cascades in these DOR-Ju.1 cells. At concentrations from 10^{-11} to 10^{-7}, deltorphin and DADLE have been shown to elevate $[Ca^{2+}]_i$ [14]. DADLE also reduced cAMP production by 70% (IC_{50} of approximately 10^{-11} M). In addition, recent studies have shown that stimulating DOR-Ju.1 T cells with deltorphin or DADLE activated the mitogen-activated protein kinases (MAPKs) ERKs 1 and 2.* Thus, DORs affect intracellular signal cascades that are known to mediate T cell activation, which is pivotal to the expression of HIV-1.

To determine whether activating DORs would modulate HIV-1 propagation, DOR-Ju.1 cells were treated with the δ agonists benzamide, 4-[[2,5-dimethyl-4-(2-propenyl)-1-piperazinyl](3-methoxyphenyl)methyl]-*N*-,[2*S*-[1(*S**),-2α,5β]]-(9Cl) (SNC-80) or deltorphin prior to the addition of HIV-1. Cells were cultured for 10 days, and p24 antigen production, an index of HIV expression, was measured in culture supernatants. These studies demonstrated that DOR agonists can substantially reduce the propagation of HIV-1 in the DOR-Ju.1 T cell model.

MATERIALS AND METHODS

Reagents

Deltorphin was obtained from Multiple Peptide Systems. Naltrindole and SNC-80 were provided by Dr. Portoghese (University of Minnesota) and Dr. Rice (National Institutes of Health), respectively.

* Shahabi NA, Daaka Y, McAllen K and Sharp BM, Manuscript submitted for publication.

Transfection and Identification of Jurkat Cells Expressing DOR

The coding sequence of murine-Flag-DOR (from Dr. M. von Zastrow, UCSF) was cloned into the *Hin*dIII/*Xho*I sites in the REP-9 expression vector (Invitrogen). As previously described, a Jurkat cell subline, Ju.1, was transfected with Flag-mDOR pREP-9 by electroporation, and G418 drug-resistant cells were sorted by flow cytometry for Flag-positive cells using M2 anti-Flag mAb (International Biotechnologies; stably transfected DOR-positive cells are designated DOR-Ju.1) [14].

Addition of DOR Ligands and HIV-1 Infection

The HIV-1 isolate used to infect DOR-Ju.1 cells was derived from peripheral blood mononuclear cells of an asymptomatic patient, as previously described [13]. In brief, DOR-Ju.1 cells (1×10^6 cells/well) were pretreated with DOR ligands for 24 hr and then were infected at a multiplicity of infection of 0.02. After extensive washing, equivalent amounts of DOR ligands were again added to the cells, and incubations were continued for 10 days; then p24 core protein levels (a marker of HIV-1 expression) were measured on the supernatants, as previously described [13]. No concentration of the DOR ligands used had an effect on cell viability, as assessed by trypan blue dye exclusion. In preliminary studies, the production of p24 antigen depended on both the number of cells seeded at time 0 and the time in culture. At 1×10^6 cells/well, the p24 antigen levels (pg/mL) were: <30 at day 0, 127 ± 13 at day 4, 522 ± 22 at day 7, and 1126 ± 30 at day 10 post-infection. Wells initially seeded with 0.05×10^6 or 0.25×10^6 cells had p24 levels of 55 ± 7 and 497 ± 7 pg/mL, respectively, at day 10.

Statistical Analysis

Where appropriate, data are expressed as means ± SEM. For comparisons of multiple group means, ANOVA was performed with Scheffe's F-test for post hoc analyses.

RESULTS AND DISCUSSION

Figure 1A shows that concentrations of SNC-80 between 10^{-13} and 10^{-9} M significantly reduced the levels of p24 antigen by 50–65% ($F = 7.8$; $P = 0.0002$). As reported with other immune responses to opiates [6, 15], higher concentrations of SNC-80 (10^{-7} M) were ineffective. Figure 1B shows that naltrindole at concentrations of 0.1 and 10 pM alone had no effect on p24 levels, whereas it blocked the response to SNC-80 (10^{-9} M). At 10 pM, naltrindole abolished the effect of SNC-80; 0.1 pM naltrindole produced a partial blockade; however, the response to SNC-80 was still significantly less ($P < 0.05$) than that of the control medium.

To investigate whether DOR peptidergic ligands would

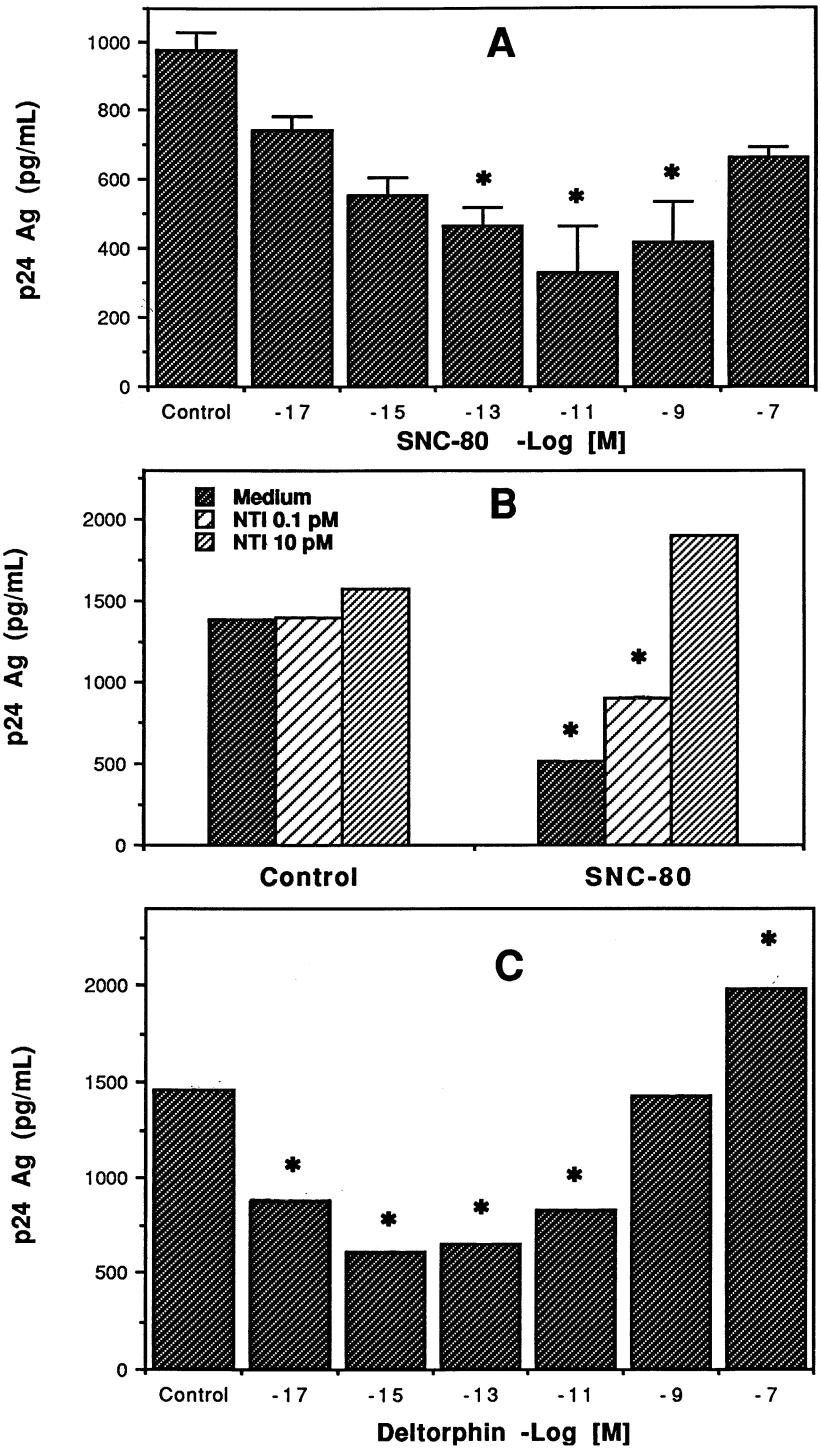

FIG. 1. Effects of SNC-80 (panel A), naltrindole (NTI) alone or 30 min prior to 10^{-9} M SNC-80 (panel B), and deltorphin (panel C) on p24 antigen levels in supernatants of DOR-Ju.1 cells infected with HIV-1. Cells were treated with DOR agonists for 24 hr, infected for 16 hr, and washed extensively; agonists were re-added, and supernatants were obtained on day 10. Data are means ± SEM, N = 4 from two separate experiments in panel A. Panels B and C show mean values for two experiments. The duplicate values for each treatment in panel B were (corresponding to the bars shown from left to right): 1430, 1350; 1421, 1385; 1567, 1579; 481, 520; 884, 912; and 2062, 1742. For panel C, the duplicate values were: 1450, 1482; 842, 915; 615, 595; 682, 621; 806, 861; 1541, 1326; and 2093, 1872. *P < 0.05, compared with respective controls.

exert a similar anti-HIV-1 effect on acutely infected cells, the selective DOR ligand deltorphin was tested. Figure 1C indicates that deltorphin also inhibited the production of p24 antigen. Concentrations of deltorphin between 10^{-17} and 10^{-11} M significantly reduced p24 antigen levels by 40–60% ($F = 68.2$; $P = 0.0001$). By 10^{-9} M, deltorphin was no longer inhibitory, and 10^{-7} M significantly enhanced p24 levels (by 30% above control). Such direct biphasic effects of opiates have been observed with cells involved in host defense and immunity [4]. Moreover, concentration-dependent effects at sub-picomolar concentrations also have been reported. DADLE significantly reduces forskolin-stimulated cAMP production in these DOR-Ju.1 T cells at picomolar concentrations [14]. Concentrations of deltorphin in the picomolar range also inhibit anti-CD_3-ε-induced proliferation and interleukin-2 production by highly purified murine splenic T cells. Deltorphin at 10^{-11} M significantly inhibits the proliferation of $CD4^+$ T cells and at 10^{-13} M is sufficient to inhibit $CD8^+$ T cells [4]. Deltorphin at 10^{-11} M enhances the

production of interleukin-2, whereas concentrations in the range of 10^{-9}–10^{-7} M are inhibitory [4].

Activation of both κ- and μ-opioid receptors (KORs, MORs) has been shown to affect HIV propagation in other systems. Both dynorphin A (1–13) and benzeneacetamide,3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-, *trans*- (9Cl) (U50,488), a KOR-selective agonist, enhance the production of p24 antigen by cocultures of normal human fetal brain cells with chronically HIV-infected promonocytes (U1 cell line) [15]. Dynorphin was effective at 10^{-15} M with peak stimulation at 10^{-13} M and no effect at 10^{-7} M. Similar to the observations we have made with SNC-80 in DOR-Ju.1 cells, U50,488 was effective in brain cocultures at concentrations between 10^{-13} and 10^{-9} M and ineffective at higher concentrations. Morphine has been shown to promote the growth of HIV-1 in human peripheral blood mononuclear cell cocultures at concentrations between 10^{-15} and 10^{-9} M, with peak effects at 10^{-12} M [13]. These effects are stereospecific and inhibited by the MOR antagonist, β-funaltrexamine.

In summary, this is the first report, to our knowledge, showing that opioid receptors can suppress the expression of HIV-1 in T cells. In contrast to the stimulatory effects of MORs, activation of T cell DORs appeared to inhibit HIV-1 expression in acutely infected $CD4^+$ T cells in the DOR-Ju.1 model. This suppression was seen with an alkaloid-like agonist (SNC-80) and with a peptide (deltorphin). Peak effects occurred in the picomolar range, as observed with other immunomodulatory effects of opiates. These findings suggest that selective adjuvant opioid immunotherapy may enhance the antiviral approach to HIV-1 infection.

References

1. Linner KM, Beyer HS and Sharp BM, Induction of the messenger ribonucleic acid for proenkephalin A in cultured murine CD4-positive thymocytes. *Endocrinology* **128:** 717–724, 1991.
2. Saravia F, Ase A, Aloyz R, Kleid MC, Ines M, Vida R, Nahmod VE and Vindrola O, Differential posttranslational processing of proenkephalin in rat bone marrow and spleen mononuclear cells: Evidence for synenkephalin cleavage. *Endocrinology* **132:** 1431–1437, 1993.
3. Linner KM, Quist HE and Sharp BM, Met-enkephalin-containing peptides encoded by proenkephalin A mRNA expressed in activated murine thymocytes inhibit thymocyte proliferation. *J Immunol* **154:** 5049–5060, 1995.
4. Shahabi NA and Sharp BM, Anti-proliferative effects of δ opioids on highly purified $CD4^+$ and $CD8^+$ murine T-cells. *J Pharmacol Exp Ther* **273:** 1105–1113, 1995.
5. Martin-Kleiner I, Osmak M and Gabrilovac J, Regulation of NK cell activity and the level of the intracellular cAMP in human peripheral blood lymphocytes by Met-enkephalin. *Exp Med (Berl)* **192:** 145–150, 1992.
6. Shahabi NA, Heagy W and Sharp BM, β-Endorphin enhances concanavalin A-stimulated calcium mobilization by murine splenic T-cells. *Endocrinology* **137:** 3386–3393, 1996.
7. Chung LF, Chuang TK, Killam KF, Chuang AJ, Kung HF, Yu L and Chuang RY, Delta opioid receptor gene expression in lymphocytes. *Biochem Biophys Res Commun* **202:** 1291–1299, 1994.
8. Wick MJ, Minnerath SR, Roy S, Ramakrishnan S and Loh HH, Differential expression of opioid receptor genes in human lymphoid cell lines and peripheral blood lymphocytes. *J Neuroimmunol* **64:** 29–36, 1996.
9. Miller B, Delta opioid receptor expression is induced by concanavalin A in $CD4^+$ T-cells. *J Immunol* **157:** 5324–5328, 1996.
10. Sharp BM, Shahabi N, McKean D, Li MD and McAllen K, Detection of basal levels and induction of δ opioid receptor mRNA in murine splenocytes. *J Neuroimmunol* **78:** 198–202, 1997.
11. Stefano GB, Melchiorri P, Negri L, Hughes TK Jr and Scharrer B, [D-Ala^2]Deltorphin I binding and pharmacological evidence for a special subtype of δ opioid receptor on human and invertebrate immune cells. *Proc Natl Acad Sci USA* **89:** 9316–9320, 1992.
12. Carr DJJ, Kim C-H, deCosta B, Jacobson AE, Rice KC and Blalock JE, Evidence for a δ-class opioid receptor on cells of the immune system. *Cell Immunol* **116:** 44–51, 1988.
13. Peterson PK, Sharp BM, Gekker G, Portoghese PS, Sannerud K and Balfour HH Jr, Morphine promotes the growth of HIV-1 in human peripheral blood mononuclear cell cocultures. *AIDS* **4:** 869–874, 1990.
14. Sharp BM, Shahabi NA, Heagy W, McAllen K, Bell M, Huntoon C and McKean DJ, Dual signal transduction through δ opioid receptors in a transfected human T-cell line. *Proc Natl Acad Sci USA* **93:** 8294–8299, 1996.
15. Chao CC, Gekker G, Hu S, Sheng WS, Portoghese PS and Peterson PK, Upregulation of HIV-1 expression in cocultures of chronically infected promonocytes and human brain cells by dynorphin. *Biochem Pharmacol* **50:** 715–722, 1995.