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UPREGULATION OF HIV-1 EXPRESSION IN COCULTURES OF CHRONICALLY INFECTED PROMONOCYTES AND HUMAN BRAIN CELLS BY DYNORPHIN

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Abstract—Using cocultures of human fetal brain cells and a chronically human immunodeficiency virus-1 (HIV-1)-infected promonocytic line U1, we investigated the effect of dynorphin, an endogenous opioid peptide found in the CNS, on upregulation of HIV-1 expression. Dynorphin and the synthetic κ receptor agonist U50,488 promoted HIV-1 expression with a bell-shaped concentration–response relationship in which maximal effects were observed at 10^{-13} and 10^{-11} M, respectively. Pretreatment for 30 min with the κ receptor antagonist nor-binaltorphimine completely blocked the stimulatory effect of dynorphin and U50,488. The involvement of cytokines on HIV-1 expression was tested. Dynorphin-induced upregulation of HIV-1 in the cocultures was largely blocked by antibodies to tumor necrosis factor (TNF)-α and interleukin (IL)-6 but not by antibodies to IL-10. Also, dynorphin stimulated TNF-α and IL-6 in the brain cell cultures at both mRNA and protein levels, suggesting the involvement of these cytokines in opioid-induced HIV-1 expression. These findings suggest that endogenous opioid peptides such as dynorphin may have an immunomodulatory function in the CNS and could act as a cofactor in the neuropathogenesis of HIV-1.

Key words: dynorphin; opioids; human immunodeficiency virus-1; neuropathogenesis; cytokines; microglia

The pathogenesis of HIV-1§-associated encephalopathy is incompletely understood. A substantial body of evidence, however, suggests that cells of the mononuclear phagocyte system play a central role in the neuropathogenesis of this virus [1]. First, blood monocytes appear to serve as a vehicle for HIV-1 entry into the CNS [2], and infected monocytes have been shown to produce substances that are neurotoxic [3, 4]. Second, microglial cells, the "resident macrophages" of the brain, harbor HIV-1 [1, 5, 6], and when activated, these cells release cytokines that are neurotoxic [7] and also can upregulate HIV-1 expression in chronically infected cells [8, 9].

To date, few studies have evaluated agents that potentially could enhance HIV-1-induced encephalopathy. Due to their immunomodulatory activity, opiates have been proposed to operate as a cofactor in the acquired immunodeficiency syndrome [10, 11]. Recently, we investigated the effect of the opiate alkaloid morphine on HIV-1 expression in a chronically infected promonocyte line U1, using a coculture assay with LPS-stimulated human fetal brain cells [12]. Morphine was found to amplify the upregulation of viral expression in this coculture system by a mechanism that involved enhanced production of TNF-α by LPS-stimulated microglial cells present within the human brain cell cultures [12]. Since LPS is unlikely to be relevant to HIV-1-induced encephalopathy, in the present study we developed a human brain and U1 cell coculture system that does not require LPS for upregulation of HIV-1 expression. Using this coculture assay, we investigated the effect of dynorphin A (1–13), an endogenous opioid peptide found in the brain [13], on HIV-1 expression.

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§ Abbreviations: Ag, antigen; GADPH, glyceraldehyde 3-phosphate dehydrogenase; HIV-1, human immunodeficiency virus-1; IL, interleukin; LPS, lipopolysaccharide; nor-BNI, nor-binaltorphimine; RT–PCR, reverse transcription–polymerase chain reaction; and TNF, tumor necrosis factor.

MATERIALS AND METHODS

Reagents. Dynorphin A (1–13) was obtained from Peninsula Laboratories, Belmont, CA. U50,488 was a gift of The Upjohn Co. (Kalamazoo, MI), and nor-BNI, a highly selective κ opioid receptor antagonist [14], was provided by P. S. Portoghese (University of Minnesota). Polyclonal antibodies (goat) specific to IL-6, IL-10 and TNF-α were obtained from R & D Systems, Inc. (Minneapolis, MN). LPS (0111:B4), polymyxin B, and L-leucine methyl ester were purchased from the Sigma Chemical Co. (St. Louis, MO).

Brain and U1 cell coculture assay system. Human fetal brain tissue was obtained from 16- to 22-week

old aborted fetuses under a protocol approved by our institutional Human Subjects Research Committee. Cultures were prepared using a previously described method [12]. On day 14, neuronal cells in these cultures are present in clusters and as single cells with interspersed microglia and oligodendrocytes, growing on a confluent supporting layer of astrocytes. At the time brain cell cultures were used in experiments, they contained approximately 50–60% astrocytes (identified by anti-glial fibrillary acid protein antibody staining; DAKO, Carpinteria, CA), 35–40% neurons (identified by anti-neuron specific enolase staining; Polysciences, Inc., Warrington, PA), 3–5% microglial cells (stained with anti-CD68 antibodies, DAKO), and 1–2% oligodendrocytes (stained with anti-galacto-cerebroside antibodies; Boehringer Mannheim Corp., Indianapolis, IN).

The chronically HIV-1-infected human pro-monocytic cell line U1, provided by the National Institute of Allergy and Infectious Diseases (Bethesda, MD), was cultured as previously described [12].

Experimental protocol. Brain cell cultures were incubated in 24-well plates in culture medium [DMEM (Sigma) with 10% heat-inactivated fetal bovine serum (HyClone Laboratories, Logan, UT)] or in medium containing the indicated concentrations of reagents for various times prior to the addition of 2×10^3 U1 cells. Preliminary experiments demonstrated that by day 4 of incubation, coculture supernatants consistently contained readily measurable levels of HIV-1 p24 Ag as a reflection of viral expression, whereas supernatants from U1 cells that were cultured alone for 4 days had barely detectable amounts of p24 Ag. In one experiment, to eliminate microglial cells, brain cell cultures were treated with L-leucine methyl ester (10 mM) for 24 hr and washed prior to the addition of dynorphin. As previously described [15], in preliminary experiments we found that L-leucine methyl ester reduced the viability of microglial cells by approximately 70% (by trypan blue dye exclusion criteria), whereas viability of astrocytes and neuronal cells was unaffected by L-leucine methyl ester under this treatment condition. Viability of U1 cells after a 5-day exposure to this agent was also unaffected by this treatment (>95% U1 cells excluded trypan blue).

HIV-1 Ag assay. HIV-1 Ag levels were measured using an enzyme-linked immunosorbent assay that detects mainly HIV-1 p24 Ag (Abbott Laboratories, North Chicago, IL), as previously described [12]. A standard curve derived from known amounts of p24 Ag was used to quantify the Ag levels in culture supernatants.

Cytokine determinations. To study the effect of dynorphin on IL-6 and TNF-α release from human brain cell cultures, cell cultures were treated with 10^{-13} M dynorphin (the maximal effective concentration), and supernatants were harvested at indicated times for measurements of IL-6 and TNF-α. IL-6 levels were determined using the IL-6-dependent B9 hepatoma cell line, as previously described [12]. TNF-α levels in brain cell culture supernatants were determined by a standard L929 cytotoxicity assay [12].

RT–PCR analysis. To investigate whether treatment of human brain cells with dynorphin stimulated IL-6 and TNF-α mRNA expression, brain cell cultures were treated with medium or medium containing 10^{-11} or 10^{-13} M dynorphin for 3 hr, an optimal time for evaluating IL-6 and TNF-α mRNA expression [16]. Total RNA was then isolated, and RT–PCR was carried out followed by agarose gel electrophoresis, as previously described [16]. Reverse transcription of 1 μg RNA was performed using oligo $d(T)_{12-18}$ primer (Pharmacia, Piscataway, NJ). The reaction mixture was incubated at 42° for 60 min followed by termination at 95° for 5 min in a programmable Tempcycler (Coy Corp., Ann Arbor, MI). The cDNA was stored at −80°.

Amplification of IL-6, TNF-α, or GADPH cDNA was performed in the presence of TaqStart antibody (Clontech, Palo Alto, CA)/Taq DNA polymerase (Promega) mixture, as previously described [16]. The mixture was subjected to PCR with each cycle consisting of 94° for 45 sec, 65° for 45 sec and 72° for 90 sec. An aliquot of PCR product was loaded in 1.5% agarose gel for electrophoresis, and the amplified DNA fragments were visualized with ethidium bromide stain. The TNF-α primer sets were 5′-CAGAGGGAAGAGTTCCCCAG-3′ (sense) and 5′-CCTTGGTCTGGTAGGAGACG-3′ (anti-sense, Perkin Elmer Co., Norwalk, CT). The IL-6 primer sets were 5′-ATGAACTCCTTC-TCCACAAGCGC-3′ (sense) and 5′-GAAGAGCC-CTCAGGCTGGACTG-3′ (anti-sense, Stratagene, La Jolla, CA). The GADPH primer sets were 5′-C CACCCATGGCAAATTCCATGGCA-3′ (sense) and 5′-TCTACACGGCAGGTCAGGTC-CACC-3′ (anti-sense, Stratagene). The sizes of the amplified fragments for TNF-α, IL-6, and GADPH were 325, 628, and 600 bp, respectively.

Statistical analysis. Data were expressed as means ± SEM. Student's *t*-test was used to compare differences between the means of two groups. Analysis of variance followed by Fisher's F-test was used to compare the differences among the means of multiple groups.

RESULTS

HIV-1 expression in human brain and U1 cell cocultures. The stimulatory effect of fetal human brain cells on the upregulation of HIV-1 expression in U1 cells is shown in Fig. 1. After 4 days of incubation, the constitutive expression of HIV-1 in U1 cells that were cultured alone was minimal (supernatants of these cultures contained only 81 ± 12 pg/mL p24 Ag, N = 16). When cocultured in the presence of human brain cells, however, viral expression was increased almost 6-fold (coculture supernatants contained 475 ± 94 pg/mL p24 Ag). Although considerable variation was observed in the stimulatory effect by different human brain cell culture specimens, in every experiment the amounts of HIV-1 p24 Ag found in the coculture supernatant exceeded ($P < 0.01$) that found in the corresponding U1 cell culture supernatant (Fig. 1).

To test whether culture medium was contaminated with endotoxin, which was responsible for the small

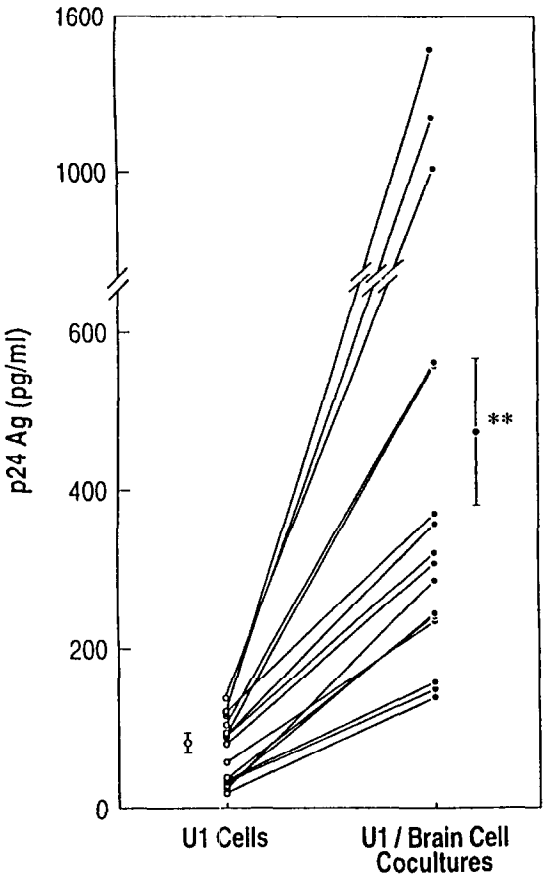

Fig. 1. Effect of human brain cells on upregulation of HIV-1 expression in U1 cells. U1 cells were incubated in medium alone or with human brain cells for 4 days. Supernatants were then harvested and assayed for HIV-1 p24 Ag levels. Data from 16 separate experiments using different brain cell specimens are given as individual values; simultaneously assayed U1 cell cultures (○) and U1/brain cell cocultures (●) are indicated by connecting lines. Data are means ± SEM values for both groups. Key: (**) $P < 0.01$ vs U1 cell culture group.

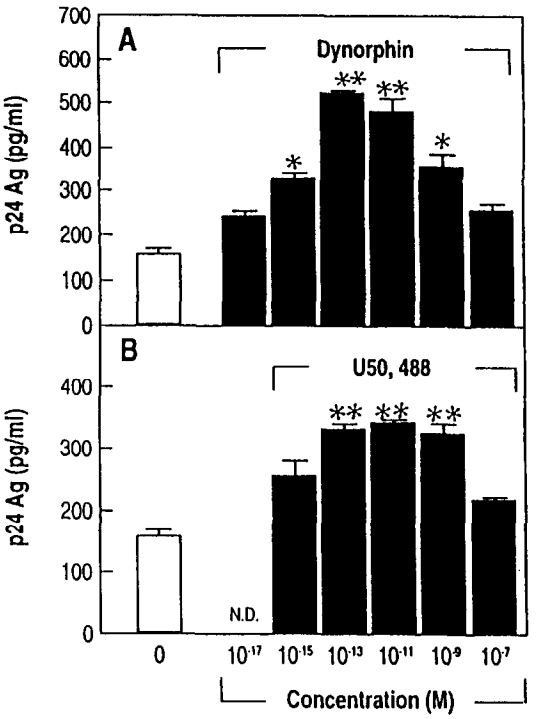

Fig. 2. Effect of κ opioid receptor agonists on HIV-1 expression in human brain and U1 cell cocultures. Human brain cells were cultured in medium containing indicated concentrations of dynorphin or U50,488 for 24 hr prior to constituting cocultures with U1 cells. After 4 days, coculture supernatants were assayed for HIV-1 p24 Ag. Data are means ± SEM of triplicates and are representative of three experiments. ND = not determined. Key: (*) $P < 0.05$ and (**) $P < 0.01$ vs control group.

amount of p24 Ag detected in the control (non-treated) cultures, an antibiotic, polymyxin B, which binds LPS, was added to the cocultures. Incorporation of 5 μg/mL polymyxin B in the human brain and U1 cell cocultures had no discernible effect on HIV-1 expression (280 ± 25 pg/mL p24 Ag vs 307 ± 25 pg/mL p24 Ag in control cocultures lacking polymyxin). When polymyxin was added to human brain and U1 cell cocultures that had been stimulated with LPS (1 pg/mL), the enhanced expression of HIV-1 (407 ± 21 pg/mL p24 Ag vs control 307 ± 25 pg/mL p24 Ag) was blocked completely (271 ± 21 pg/mL p24 Ag). LPS has been shown previously to have no direct stimulatory effect on HIV-1 replication in purified U1 cell cultures [12]. Thus, the upregulation of HIV-1 expression in the human brain and U1 cell coculture assay system used in this study is LPS independent.

Effect of κ opioid receptor agonists on HIV-1 expression in human brain and U1 cell cocultures. To investigate the effect of κ opioid receptor agonists on HIV-1 expression in human brain and U1 cell cocultures, brain cell cultures were treated with various concentrations of dynorphin A-(1–13) or U50,488 (the synthetic κ opioid receptor agonist) for 24 hr prior to constituting cocultures with U1 cells. Both dynorphin and U50,488 stimulated HIV-1 expression with a bell-shaped concentration–response effect (Fig. 2). Maximal enhancement of viral expression was observed at 10^{-13} M dynorphin (a 3.4-fold increase over control) and 10^{-11} M U50,488 (a 2.3-fold increase over control). Incorporation of polymyxin B (5 μg/mL) into cocultures of κ opioid receptor agonist-treated cocultures had no discernible effect on the increased HIV-1 expression observed in these cocultures (data not shown), suggesting an effect independent of LPS contamination. Neither dynorphin nor U50,488, at concentrations ranging from 10^{-17} to 10^{-7} M, had any direct stimulatory effect on the constitutive HIV-1 expression in purified U1 cell cultures (data not shown). Taken together, these findings indicate that dynorphin and U50,488 upregulate HIV-1 expression in human brain and U1 cell cocultures

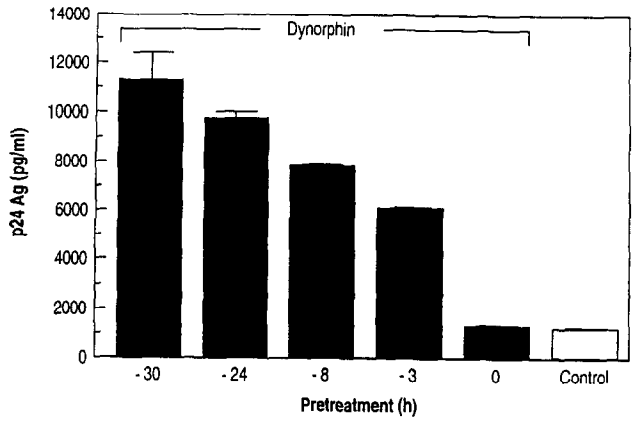

Fig. 3. Effect of duration of dynorphin treatment of human brain cells on HIV-1 expression. Prior to constituting human brain and U1 cell cocultures, human brain cell cultures were incubated for indicated times in medium containing dynorphin (10^{-13} M). Control cocultures contained human brain cells incubated in medium alone for 24 hr prior to adding U1 cells. Data are means ± SEM of triplicates and are representative of two separate experiments.

via an LPS-independent mechanism that involves participation of the brain cells.

To investigate whether the stimulatory effect of dynorphin on HIV-1 expression requires pretreatment of human brain cells, brain cell cultures were exposed to dynorphin for various periods of time prior to constituting cocultures with U1 cells. As shown in Fig. 3, the stimulatory effect of dynorphin is time dependent with marked enhancement observed after a 3-hr treatment of brain cell cultures. Absence of a stimulatory effect was seen when dynorphin was added at the same time the cocultures were constituted (Fig. 3). By way of contrast, the upregulation of HIV-1 expression induced by LPS (1 pg) did not require pretreatment of human brain cell cultures prior to constituting cocultures (data not shown).

Involvement of κ opioid receptors. To study whether dynorphin and U50,488 induced HIV-1 expression in human brain and U1 cell cocultures via κ opioid receptors, brain cell cultures were treated with the κ-selective opioid receptor antagonist nor-BNI (10^{-13} M) prior to exposure to either dynorphin (10^{-13} M) or U50,488 (10^{-11} M). While nor-BNI had no effect on HIV-1 expression in control cocultures, the stimulatory effects on HIV-1 expression of both dynorphin and U50,488 were blocked completely by the κ opioid receptor antagonist (Fig. 4). This finding suggests that a critical initial binding of dynorphin to κ opioid receptors in brain cells is required in order to stimulate subsequent upregulation of HIV-1 expression in U1 cells.

Involvement of cytokines. The upregulation of HIV-1 expression in U1 cells is known to be up- or downregulated by a number of cytokines [8]. We next investigated the involvement of cytokines in dynorphin-induced upregulation of HIV-1 expression. We reasoned that if the mechanism by which dynorphin induces HIV-1 expression involves enhanced release of a cytokine that upregulates

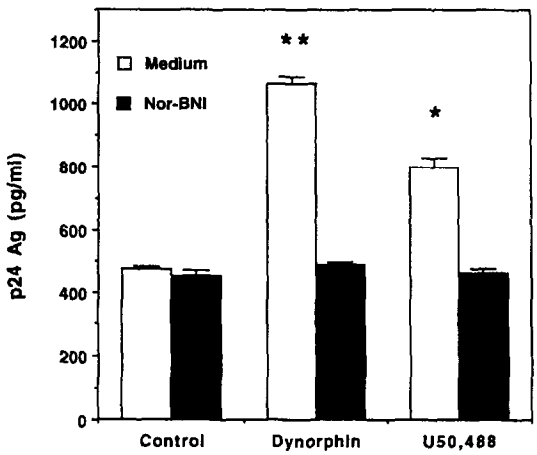

Fig. 4. Blockade of dynorphin- and U50,488-induced κ opioid receptor HIV-1 upregulation by nor-BNI. Brain cell cultures were treated with medium alone (control) or nor-BNI (10^{-13} M) for 30 min, and after washing, cultures were incubated for an additional 24 hr in medium or medium containing dynorphin (10^{-13} M) or U50,488 (10^{-11} M). Cocultures with U1 cells were then constituted, and supernatants were assayed for HIV-1 p24 Ag after 4 days of incubation. Data are means ± SEM of triplicates and are representative of three experiments. Key: (*) $P < 0.05$ and (**) $P < 0.01$ vs control value.

HIV-1, or conversely if dynorphin suppressed the release of a cytokine that downregulates HIV-1, then antibodies to such a cytokine should alter the stimulatory effect of dynorphin. Thus, prior to adding dynorphin, antibodies (10 μg/mL) specific to several cytokines that are known to affect HIV-1 expression in U1 cells were added to human brain cell cultures. The concentration of antibody selected (10 μg/mL) blocked the bioactivity of 10 ng/mL of

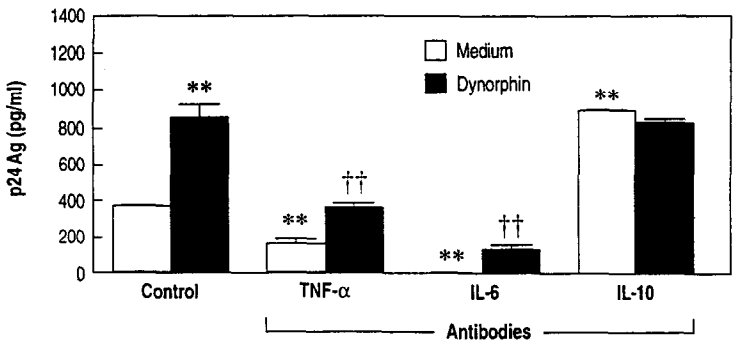

Fig. 5. Effect of antibodies to specific cytokines on dynorphin-upregulated HIV-1 expression in human brain and U1 cell cocultures. Human brain cell cultures were treated with antibodies specific to indicated cytokines or control antibody for 30 min prior to adding medium or medium containing dynorphin (10^{-13} M). After 24 hr of incubation, cocultures with U1 cells were constituted, and supernatants were assayed for HIV-1 p24 Ag levels on day 4 of culture. Data are means ± SEM of triplicates and are representative of three separate experiments. Key: (**) $P < 0.01$ vs medium control cultures and (††) $P < 0.01$ vs corresponding dynorphin control cultures.

the respective cytokines (data not shown). Antibodies to TNF-α and IL-6 each inhibited ($P < 0.01$) HIV-1 expression in control cocultures and also reduced significantly ($P < 0.01$) dynorphin-induced upregulation of HIV-1 (Fig. 5). In contrast, antibody to IL-10 markedly enhanced HIV-1 expression in control cultures while this cytokine had no effect on dynorphin-upregulated HIV-1 expression (Fig. 5). Since the enhancing effect of dynorphin was blocked almost completely by antibodies to TNF-α and IL-6, these findings suggest that TNF-α and IL-6 are involved in the stimulatory effect of dynorphin on HIV-1 replication in human brain and U1 cell cocultures.

Effect of dynorphin on TNF-α and IL-6 release. To investigate the effect of dynorphin on cytokine release from human brain cells, fetal human brain cell cultures were treated with dynorphin (10^{-13} M) or medium alone, and supernatants were removed at different time intervals over the ensuing 5 days for measurements of IL-6 and TNF-α. When compared with brain cell cultures incubated in medium alone (control), the dynorphin-treated cultures released greater ($P < 0.05$) amounts of TNF-α after an 8-hr incubation and of IL-6 after a 3-day incubation (Fig. 6). The constitutive release of TNF-α was relatively low (<3 pg/mL), while the release of IL-6 was higher (approximately 45 pg/mL). These findings support the hypothesis that dynorphin induces enhanced release from brain cells of these two cytokines, both of which are known to upregulate the expression of HIV-1 in U1 cells [8].

RT-PCR analysis. To evaluate the stimulatory effect of dynorphin on TNF-α and IL-6 gene expression, brain cell cultures were treated with medium or medium containing either 10^{-11} or 10^{-13} M dynorphin for 3 hr followed by RT-PCR analyses of TNF-α and IL-6 mRNA. Dynorphin markedly enhanced the expression of both IL-6 and TNF-α mRNA, while GADPH mRNA expression (control) was unaffected by dynorphin treatment (Fig. 7).

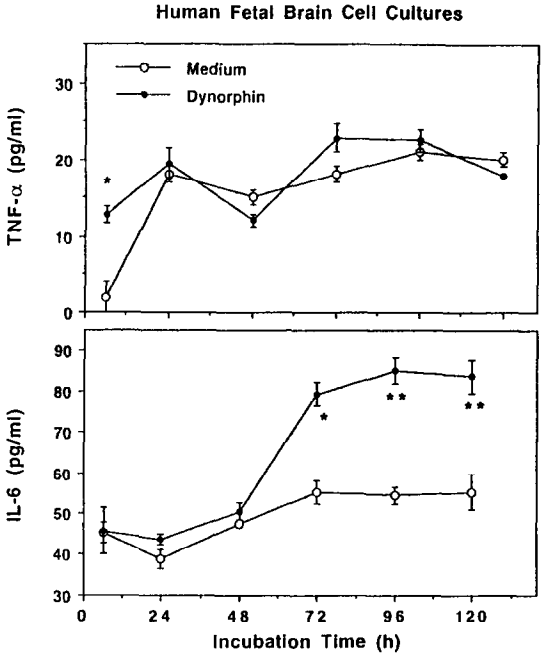

Fig. 6. Effect of dynorphin on cytokine release from human brain cells. Brain cell cultures were incubated in medium alone or medium containing dynorphin (10^{-13} M). At indicated times, supernatants were assayed for IL-6 and TNF-α levels. Data are means ± SEM of triplicates and are representative of three experiments. Key: (*) $P < 0.05$ and (**) $P < 0.01$ vs corresponding control (medium) value.

Involvement of microglial cells. Since activated human microglial cells are known to produce IL-6 and TNF-α [17], we next investigated the participation of these cells (which comprise about 3–5% of the total brain cell population in the brain cell cultures) in

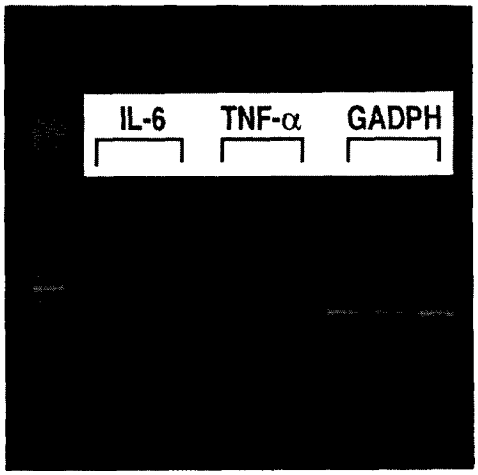

Fig. 7. Effects of dynorphin on IL-6 and TNF-α mRNA induction. Human brain cell cultures were treated with medium or medium containing indicated concentrations of dynorphin for 3 hr. Total RNA was harvested followed by RT–PCR analysis (32 cycles for IL-6 in lanes 2–4, 32 cycles for TNF-α in lanes 5–7, and 22 cycles for GADPH in lanes 8–10). Lane 1: 100 bp ladder marker; lanes 2, 5, and 8: medium control; lanes 3, 6, and 9: dynorphin (10^{-13} M); lanes 4, 7, and 10: dynorphin (10^{-11} M). Results are representative of four separate experiments.

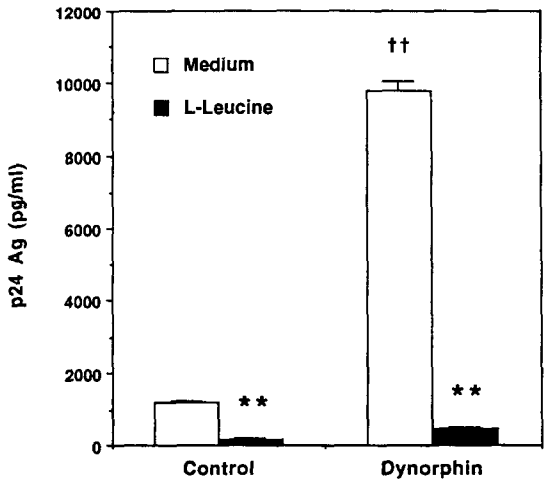

Fig. 8. Effect of L-leucine methyl ester treatment of human brain cell cultures on dynorphin-upregulated HIV-1 expression. Brain cell cultures were incubated in medium or medium containing L-leucine methyl ester (10 mM) for 24 hr before treatment with dynorphin (10^{-13} M) or incubation in medium (control) for 24 hr. Cocultures with U1 cells were then constituted, and HIV-1 p24 Ag levels were assayed in supernatants after 4 days of culture. Data are means ± SEM of triplicates and are representative of three experiments. Key: (**) $P < 0.01$ vs corresponding medium group, and (††) $P < 0.01$ vs medium control group.

dynorphin-induced upregulation of HIV-1 expression in the human brain and U1 cell cocultures. This was accomplished by treating the brain cell cultures with L-leucine methyl ester (10 mM), which selectively kills microglial cells but not other brain cell types. This treatment was found to suppress significantly ($P < 0.01$) the upregulation of HIV-1 in control cocultures and to abrogate dynorphin-stimulated viral expression (Fig. 8). This finding suggests that microglia play a key role in the upregulation of HIV-1 in this human brain and U1 cell coculture system and that dynorphin-induced upregulation of HIV-1 is also dependent mainly upon this population of glial cells.

DISCUSSION

Using a human brain and U1 cell coculture model of HIV-1 infection, dynorphin was found to induce upregulation of HIV-1 expression via a κ opioid receptor mechanism. This is the first evidence that an endogenous opioid can have a stimulatory effect on HIV-1 replication in U1 cells when these chronically infected monocytes are cultured in the presence of human brain cells. The findings in the present study with the κ opioid receptor agonists dynorphin and U50,488 differ in several regards from results of our previous studies with morphine, which by itself had no stimulatory activity but rather only amplified LPS-induced HIV-1 expression [12]. In contrast to the characteristics of LPS as a stimulus of viral expression in human brain and U1 cell cocultures [12], the stimulatory property of dynorphin was found to have a bell-shaped concentration–response characteristic, to require pretreatment of the brain cell cultures, and to be unaffected by polymyxin B. Also, while TNF-α is the key cytokine responsible for the potentiating activity of morphine [12], IL-6 appears to play a major role in dynorphin-induced upregulation of HIV-1 expression.

Dynorphin is distributed throughout the CNS [13]. Although dynorphin possesses higher affinity for κ opioid receptors, this opioid peptide also binds to μ receptors (selective for morphine-like ligands) and δ receptors (enkephalin selective) as well as to non-opioid receptor sites [18]. Unlike other endogenous opioid peptides, dynorphin has little analgesic effect in the CNS, yet it suppresses opiate withdrawal and tolerance [19]. Recent reports indicate that dynorphin plays a modulatory role in neurotransmission within the brain. Dynorphin affects long-term potentiation at the granule cell-perforant path synapse in the hippocampus [20]. Animal studies have shown that stress-induced behavior [21], food intake [22], memory [23], and glutamate receptor-mediated neurotoxicity [24] are altered by dynorphin. The findings in the present study raise the possibility that dynorphin also has an immunomodulatory effect in the brain.

Several cytokines are known to induce HIV-1 expression in U1 cell cultures [8], and certain cytokines (e.g. IL-6 and TNF-α) seem to be involved in the stimulatory effect of dynorphin, as suggested by the blockade of dynorphin's activity with antibodies to these cytokines. Although results of the present study support the involvement of

cytokines such as TNF-α and IL-6 in the stimulatory effect of dynorphin on upregulation of HIV-1 expression in this coculture system, other mechanisms may also be involved including a direct effect on the viral life cycle within the chronically infected U1 cells. Further studies are warranted to clarify the precise mechanisms involved.

Other cytokines not tested in the present study, such as IL-1, may also be involved in upregulation of HIV-1 expression [8]. Antibody to IL-6 was found to almost totally inhibit constitutive HIV-1 expression in cocultures that did not contain dynorphin. Because it has been shown that IL-1 and TNF-α induce IL-6 release in human glial cell cultures [9, 16, 17], it is possible that release of IL-6 may be the cytokine involved in the final pathway mediating HIV-1 replication in our brain and U1 cell cocultures. Interestingly, antibody to IL-10 enhanced HIV-1 expression in brain and U1 cell cocultures, suggesting that IL-10 is constitutively produced in these cultures and inhibits HIV-1 replication. Consistent with the antiviral effect of IL-10 suggested by these findings, IL-10 has been reported to be induced initially by HIV-1, which then suppresses HIV-1 expression in cultured monocytes [25]. Since dynorphin-induced HIV-1 replication was not influenced by IL-10 antibody, IL-10 appears not to be involved in the stimulatory effect of dynorphin on HIV-1 upregulation in the brain and U1 cell cocultures.

Microglial cells have been demonstrated to be the principal glial cell population of human brain that produces IL-6 and TNF-α [16, 17]. It seems likely that these brain macrophages are the main source of the increased amounts of these cytokines found in dynorphin-stimulated brain cell cultures. This hypothesis is supported by marked inhibition of HIV-1 expression in cocultures containing brain cells that had been treated with L-leucine methyl ester, a procedure that predominantly destroys microglia [15]. Substantial variation was observed in the capacities of different human fetal cell cultures of brain specimens to induce HIV-1 expression and to respond to dynorphin. This variation may be due, in part, to differences in the number of microglia or in the cytokine-producing capacity of these cells in these specimens.

Human astrocytes also have been shown to produce IL-6 [9, 17], which induces HIV-1 replication when cocultured with U1 cells [9]. It is possible that κ opioid receptor agonists (dynorphin and U50,488) also act on astrocytes, resulting in increased cytokine release and subsequent upregulation of HIV-1 expression in the brain and U1 cell cocultures. Previous studies of primary rat astrocyte cultures suggest that these glial cells do possess κ opioid receptors [26].

Although stimulation of cytokines such as IL-6 and TNF-α by dynorphin theoretically would be counterproductive in defense of the brain against HIV-1, these cytokines have been shown recently to limit the growth of *Toxoplasma gondii* in human fetal microglial cell cultures [27]. In addition to their effects on HIV-1 replication, cytokines such as TNF-α have been proposed to play a role in brain development [28]. Interestingly, endogenous opioid peptides and their receptors also have been considered to have a neurotrophic function in the developing brain [29].

While numerous studies have shown that endogenous opioid peptides alter the functional activities of somatic immune cells [30–32], little, if any, data have been provided prior to the present study regarding the effects of these peptides on the functional activities of microglia and astrocytes, the immune cells within the CNS. The results of the present study suggest that opioid receptors may also be found in microglial cells. Future studies should test this hypothesis and examine the interaction of dynorphin with δ and μ opioid receptors, assess the activity of different peptide fragments of dynorphin on cytokine production, and investigate the effects of dynorphin on other immune responses of microglial cells. Finally, the biological consequences of these interactions should be explored in animal models of inflammatory and infectious diseases of the CNS.

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