Published in final edited form as:

J Neuroimmunol. 2005 March ; 160(1-2): 68–76. doi:10.1016/j.jneuroim.2004.11.001.

The HIV-1 coat protein gp120 regulates cxcr4-mediated signaling in neural progenitor cells

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Abstract

We demonstrate that hCD4-primed gp120IIIB interacts with CXCR4 receptors expressed by postnatal mouse neural progenitor cells and elicits robust Ca^{2+} signals. The chemokine SDF-1 acted as a chemoattractant and a mitogenic stimulus for these neural progenitor cells. Although hCD4/gp120 was not able to produce chemoattraction or increase proliferation, it completely blocked the ability of SDF-1 to produce these effects. Thus, gp120 can act both as an agonist and de facto antagonist of CXCR4-mediated signaling in neural progenitor cells. It is possible that the ability of hCD4/gp120 to block SDF-1 signaling in neural progenitors may contribute to the neuropathological effects of HIV-1.

Keywords

Neural stem cells; Chemokines; HIV-1; Dementia; Migration

1. Introduction

Infection by HIV-1 produces a wide variety of neurological symptoms in adults (Narayan et al., 1995; Price et al., 1988; Kolson, 2002) and children (Tamula et al., 2003). These symptoms include depression, memory and cognitive deficits as well as a variety of motor problems and are designated “AIDS dementia” or the “HIV-1-related cognitive/motor syndrome” (Price et al., 1988; Zink et al., 2002). AIDS dementia is accompanied by a variety of neuropathological signs, including neuronal death, pallor of the white matter, dendritic damage and the occurrence of giant cells resulting from the virus-induced fusion of microglia (Kolson, 2002). In the peripheral nervous system, sensory neuropathies frequently occur in association with compromised sensory neuron function (Luciano et al., 2003). Two major mechanisms have been postulated to explain these findings. The first of these is the occurrence of HIV-1 encephalitis accompanied by many of the signs of neuro inflammatory disease. Alternatively, it appears that HIV-1 may directly compromise the functions of neurons, glia and microglia. It is likely that both of these processes contribute to the resulting clinical picture (Kaul et al., 2001; Kolson, 2002).

Furthermore, it is interesting to note that, although HIV-1-related neuropathology is of a widespread and diffuse nature and includes all of the cell types in the brain-only, microglia can harbor productively replicating virus. It has therefore been unclear how the widespread influence of the virus is disseminated. It is currently believed that infected microglia must

secrete one or more molecules that ultimately produce neurotoxicity. There are several candidates for such secreted factors (Kaul et al., 2001; Kolson, 2002), and one of these is the coat protein of HIV-1, gp120. It is well established that the cellular receptors for HIV-1 are chemokine receptors, usually CCR5, CXCR4 or both of these (Kolson, 2002). As it is now clear that microglia, glia and neurons express many types of chemokine receptors (Tran and Miller, 2003), this would provide a mechanism by which the influence of the virus could be distributed. Commensurate with this idea, several papers have demonstrated that gp120 can be neurotoxic both in vivo and in vitro and can also produce activation of astrocytes and microglia (Bezzi et al., 2001; Corasaniti et al., 2001). However, the molecular mechanisms by which gp120 produces these effects are unclear. Potentially, gp120 binding to chemokine receptors could produce chemokine antagonism, although gp120 might also act as an agonist or partial agonist in some circumstances (Popik and Pitha, 2000).

It has recently been shown that the postnatal brain is capable of forming new neurons and glia from neural progenitors and stem cells that exist in the subventricular zone, the subgranular zone of the dentate gyrus and the olfactory bulb (Kokaia and Lindvall, 2003). Although the functions of adult neurogenesis are not completely established, it is believed that this process contributes to long-term synaptic plasticity and cognitive processes and is also involved in pathological processes such as depression (Kokaia and Lindvall, 2003; Santarelli et al., 2003). Interestingly, it has been shown that HIV-1-related neurological problems tend to be more severe in children (Cooper et al., 1998). This, together with the fact that AIDS dementia commonly includes features such as depression and compromised cognition, suggests that there might be a neurogenic component to the AIDS dementia syndrome. It has recently been demonstrated that neural progenitors from both embryonic and postnatal brain express a wide variety of chemokine receptors, including CCR5 and CXCR4 (Krathwohl and Kaiser, 2004a,b; Ji et al., 2004; Peng et al., 2004; Tran et al., 2004). Indeed, deletion of the CXCR4 receptor results in a number of neuronal phenotypes that result from compromised migration of neural progenitor cells (Tran and Miller, 2003). It might therefore be supposed that gp120 could bind to neural progenitors and disrupt their normal functions. Such a mechanism might contribute to HIV-1-related neuropathology. In the present series of experiments, we have investigated this possibility and demonstrate that gp120 can elicit signaling in neural progenitor cells and alter progenitor cell proliferation and migration.

2. Materials and methods

2.1. Generation and culture of neural progenitor cells

Neurospheres from 2–4-week-old mouse tissue were cultured essentially as above, as described in Tran et al. (2004). Progenitor cells cultured in this way express nestin and can differentiate into both neurons and glia (Tran et al., 2004). Mice were sacrificed using ether anesthesia, and their brains were removed. Following dissection, cells were isolated and propagated as in Tran et al. (2004). The cell suspension was plated onto uncoated Petri flasks (BD Falcon) in neurobasal/B27/N2 media supplemented with heparin (2 µg/ml, Sigma), EGF (10 ng/ml, Sigma) and/or bFGF (10 ng/ml, BD Biosciences), as indicated. This primary culture was maintained for 6 days to allow for the development of proliferative neurospheres. At this point, the neurospheres were transferred to a 15-ml tube and centrifuged for 7 min at 700 rpm. The pellet was washed once with Hank's solution. Neurospheres were dissociated in 0.5 ml warm 0.05% trypsin with 1 mM EDTA. The cells were mixed and incubated at 37 °C for 5–6 min, with mixing every 2 min. Three milliliters of medium (NS medium) was then added, which contained: neurobasal medium (Life Technologies) 500 ml, N2 supplement (Life Technologies) 5 ml, B27 supplement (Life Technologies) 10 ml, heparin (Sigma) 2 µg/ml, L-glutamine (Life Technologies) 0.5 mM, penicillin–streptomycin (Life Technologies) 2.5 ml. The following growth factors were

added: bFGF (BD Bioscience) 10 ng/ml and/or EGF (Sigma) 10 ng/ml for each tube. The cells were mixed well by pipetting up and down at least 15 times and then transferred to 3×60-mm plates with fresh NS media containing the appropriate growth factors and incubated at 37 °C with 5% CO₂. Alternatively, single cell suspensions were used directly in experiments as indicated.

All mouse protocols were approved by the Northwestern University animal care and use committee.

2.2. Ca²⁺ Imaging

Dissociated neural progenitor cells or neurospheres were plated onto poly-D-lysine (Sigma)-coated 15-mm glass coverslips. The intracellular free calcium concentration ([Ca²⁺]_i) was measured using digital video microfluorimetry (see Meucci et al., 1998). The radiometric indicator used was fura-2 (Molecular Probes, Eugene OR). The cells were loaded with fura-2 acetoxymethyl ester (3 μM) for 30 min at room temperature and washed with a balanced salt solution (BSS; containing mM: 145 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES and 10 glucose). After loading, the cells were allowed at least 30 min following washing to allow for dye deesterification. The glass coverslips were then mounted in a custom-designed sample chamber and superfused with BSS solution at a rate of 1.5 ml/min by a gravity-fed system. Chemokines (50 nM), gp120 or hCD4/gp120 (200 pM) or other agents (e.g., AMD3100 50 nM, histamine 10 μM, ATP 10 μM or 50 mM K) were normally applied for 3 min by adding 1 ml of solution at its final concentration directly to the bath chamber after stopping the flow. Free Ca²⁺ concentration was calculated by digital video microfluorimetry using an intensified CCD camera (Hamamatsu). The camera was coupled to a microscope (Nikon Diaphot), and the data acquisition was carried out by a Pentium computer using the MetaFluor software from Universal Imaging. [Ca²⁺]_i was calculated using conventional fura-2 f340/f380 ratios.

2.3. Chemotaxis assay

For the measurement of chemotaxis using progenitor cells, Dunn chambers were obtained from Hawksley Technology (Lancing Business Park, West Sussex BN15 8TN, UK, <http://www.hawksley.co.uk/>). Dunn chambers allow for the generation of a stable chemotactic gradient and observation of cell migration in the context of this gradient (Zicha et al., 1998). Neural progenitor cells were plated as single cells onto poly-D-lysine-coated 15-mm glass coverslips and allowed to adhere in serum-free medium for 3 h. Dunn chambers were prepared by adding vacuum grease around the outer edge of the outer chamber. As a control, neurobasal media were added to both the inner and outer chambers. To create a chemokine gradient, neurobasal media were added to the inner chamber, while CXCL12/ SDF-1 (50 nM, R&D Systems) or other agent (gp120 or hCD4/gp120, 200 pM or AMD3100, 50 nM) was added to the outer chamber. After preparation of these chambers, coverslips were washed twice with neurobasal media and then placed onto the chambers, with the cells facing down into the wells, and the chambers were incubated at 37 °C. Using an inverted microscope, the number of cells were counted for eight fields each in the inner as well as the outer chamber at the time points indicated.

2.4. Proliferation assay

Neural progenitor cells were dissociated and plated as single cells onto poly-D-lysine-coated glass coverslips, and they are allowed to adhere to the coverslips overnight. On the following day, these cells are treated with SDF-1/CXCL12 (50 nM, R&D Systems) with or without other agents (gp120 or hCD4/gp120 200 pM) diluted in neurobasal/B27/N2 media containing low concentrations of EGF and FGF (1 ng/ml). After 24 h, the cells are fixed with 4% formaldehyde (Sigma) and immunostained with Ki67 (mouse IgG, 1:300, BD

Bioscience) which is a nuclear cell proliferation-associated antigen expressed in the active stages of the cell cycle. In addition, the coverslips are counterstained with Hoescht 33342 (2 µg/ml, Molecular Probes) to determine total cell numbers. To assess the percentage of cell proliferation, the number of Ki67-positive cells as well as the Hoescht-stained cells was counted for five fields each per coverslip.

2.5. Western blotting

Neurospheres were cultured for 10 days prior to changing to medium with no EGF and bFGF media (Neurobasal/B27/N2 media supplemented with 2 µg/ml heparin) for 4 h. SDF-1 (50 nM) or gp120 (200 pM) with CD4 (200 pM) was then added to the media, and cells were collected after 10 or 30 min. Cells were washed twice in PBS and then 1×10^6 cells were lysed in 100 µl lysis buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton-X 100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na₃VO₄, 1 µg/ml Leupeptin, 1 mM PMSF). Total cellular protein was measured using a protein microplate assay kit (Bio-Rad).

Polyacrylamide/SDS gels (10%) were prepared using mini-PROTEAN 3 cell (Bio-Rad) equipment. Total protein of equal quantity (25 mg/lane) was loaded onto each SDS gel, which was run at 150 V for 1.5 h. The proteins were electroblotted to PVDF membrane (Bio-Rad) in 25 mM Tris, 192 mM glycine, 20% methanol. After the electrotransfer, the membranes were blocked in 5% skimmed milk in PBS and immunostained with rabbit polyclonal antibodies against phosphorylated or nonphosphorylated p44/42, SAPK/JNK and p38 MAPK proteins (Cell Signaling) 1:1000 for 1.5 h following incubation with antirabbit IgG 1:2000 conjugated. Using the ECL detection kit (Amersham Biosciences), membranes were developed and exposed to Hyperfilm.

2.6. Reagents

Soluble hCD4 and gp120 IIIB were obtained from the NIH AIDS reagent organization. For hCD4/gp120 solutions, the two reagents were mixed at equimolar concentrations (200 pM) prior to use.

3. Results

3.1. hCD4/gp120 activates CXCR4 receptors expressed by neural progenitors

We have previously demonstrated that postnatal neural progenitor cells, cultured as neurospheres, express several types of chemokine receptors and that activation of these receptors results in an increase in $[Ca^{2+}]_i$ (Tran et al., 2004). In the present experiments, we used this paradigm to examine whether gp120 could produce chemokine-like signaling in these cells. In our experiments, we used gp120_{IIIB}, which has been widely used in studies of gp120 neurotoxicity and is selective for CXCR4 receptors (Corasaniti et al., 2001). In our experiments, the gp120 was first “primed” with soluble hCD4 to better mimic the situation in human cells, where hCD4 usually acts as a coreceptor for HIV-1 (Lee et al., 2003). As can be seen in Fig. 1, addition of hCD4/gp120 resulted in clear increases in Ca^{2+} that were of a similar size to those produced by a variety of chemokines or agonists acting at other receptors such as histamine or ATP receptors (Tran et al., 2004). hCD4 when added by itself did not produce Ca^{2+} signals. As reported previously, all neural progenitors responded to the addition of ATP and/or histamine (Tran et al., 2004). If the effects of gp120/hCD4 are produced through activation of CXCR4 receptors, the drug AMD3100 should block them. This compound is a bicyclam that acts as a CXCR4 antagonist and blocks the interaction of T-tropic strains of HIV-1 with target cells (Zhang et al., 2002). Addition of AMD3100 generally produced no effect, although occasionally small or midsize Ca^{2+} signals were noted (Fig. 2). The ability of the drug to elicit small Ca^{2+} signals presumably results from

the fact that it is actually a very weak partial agonist rather than a pure antagonist (Zhang et al., 2002). Following addition of AMD3100, the effects of both SDF-1 and of hCD4/gp120 were clearly blocked. Thus, these results demonstrate that hCD4/gp120 can interact with CXCR4 receptors expressed by neural progenitor cells and produce agonist-like signaling.

3.2. hCD4/gp120 inhibits the functions of SDF-1 in neural progenitors

It has previously been demonstrated (Tran et al., 2004) that SDF-1 has several effects on neural progenitors both in vivo and in vitro. First, SDF-1 acts as a chemoattractant for these cells, and this is important in the development of several areas of the brain (Lu et al., 2002; Bagri et al., 2002; Klein et al., 2001; Tran and Miller, 2003). In addition, SDF-1 also stimulates the proliferation of progenitors in several cases (Klein et al., 2001; Tran and Miller, 2003; Tran et al., 2004). We therefore investigated whether hCD4/gp120 could interfere with these effects of SDF-1. As can be observed in Fig. 3, SDF-1 acted as a chemoattractant for neural progenitor cells, an effect that was inhibited by AMD3100. Interestingly, although hCD4/gp120 produced Ca^{2+} transients in these cells, it did not produce chemoattractant effects. On the contrary, coapplication of hCD4/gp120 together with SDF-1 completely inhibited the effects of the chemokine. Furthermore, we have previously demonstrated that SDF-1 produces an increase in the proliferation of embryonic (E17) progenitors (Tran et al., 2004), and Fig. 3 demonstrates that this was also the case for adult progenitors. In addition, not only did hCD4/gp120 have no effect on progenitor cell proliferation, but also, as was the case with the chemotaxis experiments, hCD4/gp120 inhibited the increase in proliferation produced by SDF-1.

3.3. Interactions between SDF-1 and hCD4/gp120

The above data demonstrate that hCD4/gp120 can inhibit several of the functional effects of SDF-1 on neural progenitors, although both molecules produce CXCR4-mediated Ca^{2+} transients in these cells. As it is clear that hCD4/gp120 does not act as a receptor antagonist, like AMD3100, we wondered if it might inhibit the effects of SDF-1 by producing receptor desensitization and so act as a de facto antagonist. We therefore examined the ability of SDF-1 to produce Ca^{2+} signals after first applying hCD4/gp120. We observed that we were still able to elicit clear effects of SDF-1 under these circumstances, suggesting that desensitization had not occurred. Quantitation of these effects (Fig. 4) demonstrated that the effects of SDF-1 were not significantly diminished following treatment with hCD4/gp120. On the other hand, although we also regularly observed effects of hCD4/gp120 following prior addition of SDF-1 (Fig. 4), the effects of hCD4/gp120 were significantly diminished. These data suggest that, although both SDF-1 and hCD4/gp120 are agonists at the CXCR4 receptor, SDF-1 produces a greater degree of receptor desensitization.

To further examine the relative signaling consequences of SDF-1 and hCD4/gp120 in neural progenitors, we measured the effects of these agonists on activation of members of the MAP kinase pathway. In these experiments, we used Western blotting to monitor the appearance of the activated (phosphorylated) forms ERK 1/2, JNK and p38 MAP kinases following treatment of cells with SDF-1 or hCD4/gp120 (Fig. 5). We found that both SDF-1 and hCD4/gp120 produced clear activation of all of these enzymes over a similar time course. Thus, enzyme activation was observed at 10 and 30 min following treatment with either agonist.

4. Discussion

The fact that the CXCR4 chemokine receptor has been shown to be widely expressed by embryonic and adult neural progenitor cells and has also been shown to be important in regulating the functions of these cells suggests that HIV-1 might potentially impact

neurogenesis in both the developing and adult brain (Kao and Price, 2004; Krathwohl and Kaiser, 2004a,b; Ji et al., 2004; Ni et al., 2004; Peng et al., 2004; Tran et al., 2004). Indeed, it has become increasingly apparent that signaling by the chemokine SDF-1 plays a key role in the development of both the central and peripheral nervous systems. SDF-1 signaling has been shown to regulate the formation of the dentate gyrus (Bagri et al., 2002; Lu et al., 2002), the internal granule layer of the cerebellum (Zou et al., 1998), the migration of cortical interneurons (Stumm et al., 2003) and the formation of the dorsal root ganglia (Belmadani et al., submitted for publication).

Two major roles for SDF-1 signaling during brain development have been demonstrated. First, the chemokine is clearly involved in the migration of embryonic neural progenitor cells and their early postmitotic derivatives. Secondly, SDF-1 has been shown to act as a mitogen for embryonic neural progenitors. Both of these actions have been demonstrated in vivo and in vitro (Bagri et al., 2002; Klein et al., 2001; Lu et al., 2002; Tran et al., 2004). As we have now demonstrated, SDF-1 can also act as a chemoattractant and mitogen for postnatal neural progenitors (Fig. 3). Although it has not been demonstrated that SDF-1 regulates neurogenesis in the adult brain, the close juxtaposition of SDF-1 and CXCR4 expression observed in the adult dentate gyrus makes this very likely. In the adult, SDF-1 is expressed by dentate granule neurons, whereas CXCR4 receptors are expressed by progenitor cells in the subgranular zone (Lu et al., 2002; Stumm et al., 2002). Thus, it is possible that T-tropic (X4) versions of gp120 might alter neurogenesis at several points, and the results presented here suggest that this might indeed be the case. In addition, it has recently been demonstrated that there are fewer neural progenitors in the brains of HIV-1-infected patients (Krathwohl and Kaiser, 2004b). Furthermore, Ni et al. (2004) have shown that gp120 inhibits the chemoattractant effects of SDF-1 on human embryonic neural progenitors. Both of these results are consistent with those reported here.

It is clearly important to understand the mechanisms underlying these effects of gp120. Since treatment of cells with hCD4/gp120 inhibited both SDF-1-induced migration and proliferation, the most obvious hypothesis accounting for these observations might have been that hCD4/gp120 bound to CXCR4 receptors and acted as an antagonist of the effects of SDF-1. However, the situation is clearly more complicated. Although de facto antagonism was observed, the pharmacological mechanism underlying this effect is not completely clear.

Although it is well established that gp120 proteins derived from T-tropic strains of HIV-1 bind to CXCR4 receptors, the signaling consequences of this interaction seem quite variable (Popik and Pitha, 2000; Lee et al., 2003). In some cases, binding of gp120 appears to be able to elicit agonist-like signaling effects, although these are not always exactly the same as those produced by SDF-1. The idea that the binding of different ligands to the same receptor may have diverse signaling consequences is consistent with current views of G-protein-coupled receptor function in which different types of agonists can stabilize distinct conformations of the receptor, resulting in different types of signaling (Azzi et al., 2003; Gbahou et al., 2003). For example, normal, inverse and protean agonists acting at the same receptor can regulate different types of signaling via the MAP kinase pathway (Azzi et al., 2003; Gbahou et al., 2003). Indeed, immunological evidence strongly suggests that CXCR4 receptors can exist in different conformations (Baribaud et al., 2001). A further level of complexity may also accrue from the ability of CXCR4 receptors to dimerize, an association that may also have signaling consequences (Vila-Coro et al., 1999; Toth et al., 2004). Thus, it is possible that gp120 and SDF-1 are different types of agonists acting at the CXCR4 receptor.

In another publication addressing this subject, Lazarini et al. (2000) used primary cultures of embryonic striatal progenitors and observed that gp120 did not mimic the chemoattractant effects of SDF-1 in these cells. We concur with these findings. However, Lazarini et al. (2000) did not examine whether gp120 might actually inhibit the effects of SDF-1 signaling. We have examined this important possibility and found it to be the case. Furthermore, in a recent publication, Ni et al. (2004) also observed that gp120 inhibited SDF-1-induced chemotaxis of human embryonic progenitor cells. From the preceding discussion, it is clear that the mechanism by which the antagonistic effects of gp120 are produced cannot be due to neutral receptor antagonism. This is because hCD4/gp120 produced Ca^{2+} signals in neural progenitor cells, and these effects clearly resulted from CXCR4 activation since both the effects of SDF-1 and of hCD4/gp120 were completely blocked by AMD3100, a selective antagonist of CXCR4 receptors. Furthermore, hCD4/gp120 and SDF-1 produced similar activation of the members of the MAP kinase signaling pathway. A second possibility is that gp120 produces receptor desensitization, thereby occluding the effects of SDF-1. However, our data do not support this possibility as hCD4/gp120 did not inhibit the subsequent actions of SDF-1 observed in the functional Ca imaging paradigm. Interestingly, we observed clear differences in the ability of SDF-1 and hCD4/gp120 to desensitize CXCR4 signaling, with the chemokine producing far more profound desensitization. These data are consistent with our previous studies demonstrating that SDF-1 produces rapid internalization of CXCR4 receptors in a variety of cell types, whereas hCD4/gp120 generally does not (Bodner et al., 2003). Thus, it is likely that intracellular signaling events produced by the actions of hCD4/gp120 antagonize those produced by SDF-1. It is known that activation of CXCR4 receptors results in a very large number of signaling events (Tran and Miller, 2003). Considering that SDF-1 and hCD4/gp120 clearly interact with the CXCR4 receptor differently (Toth et al., 2004), it would not be surprising if some of their signaling consequences differed. We examined this possibility by measuring the effects of SDF-1 and hCD4/gp120 on MAP kinase signaling. Indeed, it has been previously demonstrated that hCD4/gp120 can produce activation of the p38 branch of the MAP kinase pathway (Kaul and Lipton, 1999), something that could serve to limit both chemotaxis and proliferation (Rincon and Pedraza-Alva, 2003; Vitale et al., 2004). In our studies, we also observed that hCD4/gp120 could activate p38, as well as ERK and JNK. However, as these effects were also produced by SDF-1, they cannot explain the ability of hCD4/gp120 to antagonize the actions of SDF-1. Thus, it remains possible that there are differences in the relative effects of the two agonists on other signaling pathways.

In summary, the fact that gp120 can interfere with the normal functions of SDF-1 in neural progenitor cells suggests the possibility that this may contribute to the neuropathological effects of HIV-1. Thus, these data, together with observations on the brains of HIV-1-infected individuals, leads us to suggest the hypothesis that these effects may make a contribution to the mechanisms underlying HIV-1-associated dementia. The significance of this possibility awaits further investigation (Kao and Price, 2004).

Acknowledgments

Supported by NIH grants NS043095, DA013141 and MH040165.

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Fig. 1. hCD4/gp120 increased Ca in neural progenitor cells. Sample traces illustrating the ability of hCD4/gp120 (200 pM) to increase Ca in adult neural progenitor cells in comparison to the effects three different chemokines (CXCL12/SDF-1: CCL11/eotaxin and CCL27/CTACK—all 50 nM) and agonists at other receptors (ATP, 10 μ M and histamine 10 μ M: HIS) (see also Tran et al., 2004). In this experiment, the absolute magnitude of the effects produced were (nM, mean $\pm$ S.E., $n=11$) SDF-1 (616 $\pm$ 107); eotaxin (118 $\pm$ 32); CTACK (349 $\pm$ 67); hCD4/gp120 (168 $\pm$ 40); histamine (595 $\pm$ 81); ATP (712 $\pm$ 88).

Fig. 2.

AMD3100 antagonism of the effects of SDF-1 and hCD4/gp120. Addition of AMD3100 (10 nM) produced either a small or a moderate size Ca transient, as can be seen in these two examples. However, following AMD3100 addition, the effects of either hCD4/gp120 or SDF-1 were blocked. Note that AMD3100 did not block the effects of either histamine or ATP. Following AMD3100 treatment, the magnitude of the SDF-1 response was 0 nM, and that of hCD4/gp120 was 8 ± 4 nM, $n=8$ cells taken from two separate cultures (compare with Fig. 1).

Fig. 3. hCD4/gp120 inhibited SDF-1-induced chemotaxis and proliferation of adult neural progenitor cells. (A) SDF-1 (50 nM) induced chemotaxis of dissociated adult neural progenitors, whereas hCD4/gp120 (200 pM) did not. AMD3100 (10 nM) and hCD4/gp120 both inhibited SDF-1-induced chemotaxis. Top panel illustrates mean data taken from three separate experiments from three separate cultures. Bottom panel illustrates the same data replotted as a histogram, illustrating the mean $\pm$ S.E. (B) SDF-1 increased proliferation of adult neural progenitor cells. hCD4/gp120 was ineffective in increasing proliferation but inhibited the effects of SDF-1. ** p <0.01; * p <0.05, relative to control (untreated), n =15 (five fields from each of three coverslips per condition). The experiment was performed twice (different cultures) with the same result.

Fig. 4.

Cross-desensitization between SDF-1 and hCD4/gp120. (A) Examples of Ca responses in postnatal neural progenitor cells illustrating the effects of SDF-1 added after an initial stimulus using hCD4/gp120 and (B) effects of hCD4/gp120 following an initial stimulus using SDF-1. In the experiments in panel (A), the response to hCD4/gp120 was 213 ± 43 nM, and the response to SDF-1 was 214 ± 140 nM, $n=10$. These were not significantly different ($p>0.05$). In the experiments in panel (B), the response to SDF-1 was 138 ± 33 nM, and the response to hCD4/gp120 was 47 ± 27 nM, $n=14$. $p<0.001$ was significantly different. Cells were taken from three separate cultures.

Fig. 5.

Activation of the MAP kinase pathway by SDF-1 and hCD4/gp120. Western blots of whole cell lysates from postnatal neural progenitor cultures illustrate a time-dependent activation of ERK, JNK and p38. Results are one of two experiments in each case.