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RESEARCH PAPER

Serotonin decreases HIV-1 replication in primary cultures of human macrophages through 5-HT$_{1A}$ receptors

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Background and purpose: 5-HT (serotonin) is known to be involved in neuroinflammation and immunoregulation. The human immunodeficiency virus (HIV) targets cells such as monocytes/macrophages, which colocalize with 5-HT-releasing cell types, mostly platelets. In this study, we investigated the effects of 5-HT on HIV-1-infected macrophages *in vitro*.
Experimental approach: Human macrophages cultured in serum-free medium were treated over 7 days with 5-HT at three concentrations (0.01, 1 and 100 μM) with or without agonists and antagonists of 5-HT$_{1A}$ and 5-HT$_2$ receptors. After 7 days of treatment, macrophages were infected with HIV-1/Ba-L and virus replication was monitored over 16 days and expression of proviral HIV DNA was investigated by PCR after 24 h of infection. Cell surface expression of HIV-1/Ba-L receptor (CD4) and coreceptor (CCR5) was investigated by flow cytometry. The CCR5 ligand, macrophage inflammatory protein-1α (MIP-1α), was quantified by ELISA in cell culture supernatants and MIP-1α mRNA expression was assessed by reverse transcriptase-PCR.
Key results: *In vitro,* 5-HT downregulated the membranous expression of CCR5 and led to a decrease of HIV-1 infection, probably through its action on 5-HT$_{1A}$ receptors. 5-HT (100 μM) was also able to induce overexpression of MIP-1α mRNA leading to an increase of MIP-1α secretion by human macrophages.
Conclusions and implications: The effects of 5-HT on HIV infection could be a consequence of the increase in MIP-1α concentrations and/or CCR5 receptor downregulation. These results suggest that 5-HT can inhibit the replication of HIV-1 in primary culture of human macrophages through its action on 5-HT$_{1A}$ receptors.
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Abbreviations: αCH$_3$-5-HT, α-methyl-5-HT maleate salt; 8-OH DPAT, 8-hydroxy-2-(di-*n*-propylamino)-tetralin; C_T, cycle threshold; HIV, human immunodeficiency virus; MdM, monocyte-derived macrophages; MIP-1α/CCL3, macrophage inflammatory protein-1α; RT, reverse transcriptase; SFM, serum-free media; WAY100635, *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)-ethyl)-*N*-2-pyridinylcyclohexanecarboxamide

Introduction

Macrophages are one of the major cellular targets of the human immunodeficiency virus (HIV) and one of the main viral reservoirs (Ho *et al*., 1994; Kedzierska and Crowe, 2002; Verani *et al*., 2005). Cells of the macrophage lineage play an important role in the neuropathogenesis of HIV-1 infection and contribute to HIV-induced dementia via production of proinflammatory cytokines and neurotoxins (Merrill and Chen, 1991; Smith *et al*., 2001; Guillemin *et al*., 2005a, b; Verani *et al*., 2005). The β-chemokine receptor CCR5 is the major coreceptor in conjunction with CD4 for macrophage-tropic viruses (R5 strains), such as HIV-1/Ba-L (Choe, 1998). Chemokines, cytokines and their receptors play a pivotal role in the extent of virus replication, dissemination and HIV pathogenesis (Fantuzzi *et al*., 2003; Kedzierska *et al*., 2003).

In inflammatory areas, macrophages are exposed to a variety of autocrine products released by lymphocytes and

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platelets (Jeynes *et al*., 1980; Pawlowski *et al*., 1983). Among these molecules, the inflammatory monoamine 5-HT is released by platelets (Benedict *et al*., 1986; Timmons *et al*., 1986; Endo *et al*., 1997).

The concentration of 5-HT can be regulated via its transport into monocytes and dendritic cells through activation of 5-HT uptake systems (Mossner and Lesch, 1998; Gordon and Barnes, 2003). There is evidence that 5-HT is a key mediator involved in intercellular (for example, between dendritic and T cells) signalling during inflammatory processes (O'Connell *et al*., 2006). 5-HT has been shown to play a role in the control of HIV replication in lymphocytes (Sidibe *et al*., 1996), and the inhibition of 5-HT reuptake can enhance the function of natural killer cells (Evans *et al*., 2007). Monocytes/macrophages express specific 5-HT receptors involved in the immunomodulatory effects of 5-HT (Frank *et al*., 2001; Idzko *et al*., 2004; Durk *et al*., 2005). So far, only the 5-HT_{1A} receptor (Alexander *et al*., 2007) has been described in human macrophages (Mossner and Lesch, 1998). In mouse macrophages, both 5-HT_{1A} and 5-HT_{3A} receptors have been described (Freire-Garabal *et al*., 2003; Cloez-Tayarani and Changeux, 2007). The 5-HT_{1A} receptor is coupled to G-proteins and linked to AC and PKC. A second signalling pathway activating the mitogen-activated protein (MAP) kinase is mediated by endocytosis of 5-HT_{1A} receptors and phosphorylation by G-receptor kinase (Richter *et al*., 2003).

We therefore hypothesized that 5-HT would modulate HIV infection in human monocyte-derived macrophages (MdM). In this study, we sought to understand the complex interactions associated with the colocalization of HIV cell targets, such as monocytes/macrophages, and 5-HT-releasing cells, as well as the role of 5-HT in inflammatory processes (which are involved in the control of HIV replication). We also investigated the effects of 5-HT on the production of the chemokine macrophage inflammatory protein-1α (MIP-1α or CCL3) and its impact on the p38 MAP kinase pathway. MIP-1α, a natural ligand for the HIV-coreceptor CCR5, is able to inhibit HIV entry and replication in macrophages (Capobianchi *et al*., 1998; Cocchi *et al*., 2000). The p38 MAP kinase pathway is involved in the induction of MIP-1α gene transcription in eosinophils and possibly other cells (Adachi *et al*., 2000; Wong *et al*., 2005).

Methods

Cells

Human peripheral blood mononuclear cells were isolated from healthy seronegative donors from the Centre de Transfusion Sanguine des Armées by Ficoll-Hypaque density gradient centrifugation. Monocytes were separated from peripheral blood mononuclear cells by successive adherence steps and allowed to differentiate into MdM for 7 days in macrophage-SFM (serum-free medium) cell culture medium supplemented with 1% antibiotic mixture (penicillin, streptomycin and neomycin). The expression of various cell markers, including CD14, HLA-DR, CD32, CD86 and CD163, has previously been compared between 7-day-differentiated MdM grown in macrophage-SFM and those grown in RPMI1640 cell culture medium supplemented with FCS and penicillin-streptomycin-neomycin mixture. The reagent macrophage-SFM did not alter the previously described MdM phenotypic profile (Rimaniol *et al*., 2000; Rogez *et al*., 2003). Cell surface expression of CD14, HLA-DR, CD32, CD86 and CD163 could be easily altered by MdM culture conditions (unpublished personal data).

Virus infection of macrophages and quantification of HIV replication

Monocyte-derived macrophages were infected at a multiplicity of infection of 1 with the reference macrophage-tropic HIV-1/Ba-L strain (Gartner *et al*., 1986) 7 days after the isolation of monocytes with consistent peak CCR5 expression (Gartner *et al*., 1986; Naif *et al*., 1998). This virus was prepared as described previously (Rogez *et al*., 2003).

Cell culture supernatants were collected every 3 or 4 days. HIV replication was assessed in the supernatants every 3 or 4 days by quantitative reverse transcriptase (RT) activity using the RetroSys kit (Innovagen, Lund, Sweden). Results are presented as the cumulative RT activity until the peak of HIV replication observed at day 16.

Treatment of MdM

The absence of toxicity of 5-HT, agonists and antagonist of 5-HT_{1A} and 5-HT_2 receptors were checked on human MdM in primary cultures using the colorimetric MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) assay. Dilutions of 5-HT, agonists and antagonist of 5-HT_{1A} and 5-HT_2 receptor subtypes were performed in cell culture media, and treatments were renewed every 2 or 3 days.

The higher concentration of 5-HT used in this study (100 μM) has been described previously as the amount released by activated platelets at inflammatory sites (Benedict *et al*., 1986) and has been previously used by others *in vitro* studies (Cloez-Tayarani *et al*., 2003, 2004; Freire-Garabal *et al*., 2003).

Effects of 5-HT on HIV-1 replication. Three experimental conditions were tested: (i) MdM were treated with three different concentrations of 5-HT (0.01, 1 and 100 μM) for 7 days and then infected, (ii) for the higher 5-HT concentration (100 μM), MdM were treated and infected simultaneously and (iii) MdM were infected and treated with 5-HT 3 h after infection.

Effects of 5-HT receptor agonists on HIV proviral DNA. Monocyte-derived macrophages were treated with 5-HT (0.01, 1 and 100 μM), 5-HT (100 μM) in combination with *N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)-ethyl)-*N*-2-pyridinyl-cyclohexanecarboxamide (WAY100635), (7)-8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH DPAT) or α-methyl 5-HT maleate salt (αCH_3-5-HT). 8-OH DPAT, WAY100635 and αCH_3-5-HT combinations were used at 50, 10 and 50 μM, respectively, as previously described by Abdouh *et al*. (2004). As the 5-HT_2 receptor subtype has not been clearly described on the cell surface of macrophages, we chose to use the full 5-HT_2 receptor agonist αCH_3-5-HT. After 7 days of treatment, MdM were infected for 24 h; then, the proviral DNA was quantified by PCR.

Effects of 5-HT on CD4 and CCR5 expression. Uninfected MdM were treated with 5-HT (0.01, 1 and 100 μM), WAY100635 (10 μM), 5-HT (100 μM) in combination with WAY100635 (10 μM) or 8-OH DPAT (50 μM).

Effects of 5-HT on MIP-1α synthesis. The production of MIP-1α was quantified in uninfected MdM culture supernatants after 7 days of treatment with 5-HT (100 μM), 8-OH DPAT (50 μM), WAY100635 (10 μM) or 5-HT (100 μM) in combination with WAY100635 (10 μM).

Effects of 5-HT on mRNA MIP-1α expression. The mRNA MIP-1α expression was quantified in uninfected MdM after 7 days of treatment either with 5-HT (100 μM) with or without WAY100635 (10 μM) or with 8-OH DPAT (50 μM). The p38 MAP kinase inhibitor SB 203580 was used at 10 μM concentration (Napolitano *et al*., 2004; Chien *et al*., 2005) in combination with either 5-HT (100 μM) or 8-OH DPAT (50 μM).

Quantification of proviral HIV DNA
Quantification of HIV proviral DNA was performed by PCR using an iCycler thermal cycler (Bio-Rad, Hercules, CA, USA). The *gag* (SK38-SK39) and β-globin genes were amplified by PCR, as described previously (Rogez *et al*., 2003). Cells were lysed using proteinase K and DNA was extracted using DNeasy tissue system (Qiagen, Courtaboeuf, France). After staining with ethidium bromide, specific bands were visualized on a transilluminator (UVP Life Sciences, Corston, UK) and quantified by densitometry (NIH 1.2, W Rasband; National Institutes of Health, Bethesda, MD, USA). Results are expressed as follows: $100 \times$ ((number of proviral DNA copies)/(number of β-globin gene copies/2)).

Quantification of the expression of MIP-1α mRNA
The levels of mRNA MIP-1α expression were measured using real time RT-PCR. Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Courtaboeuf, France). RNA preparation was treated with DNase-free RNase (Qiagen) and subjected to reverse transcription with oligo-dT primers (Eurobio, Courtaboeuf, France). The resulting cDNA was amplified by real-time PCR (iCycler; Bio-Rad) using GeneAmp RNA PCR (Applied Biosystems, Foster City, CA, USA) with the following specific primers:

GAPDH	Forward	5′-TCGTGGAAGGACTCATGACC-3′
	Reverse	5′-TCAGCTCAGGGATGACCTTG-3′
MIP-1α	Forward	5′-CACCCATGAGTGTGAGCAGA-3′
	Reverse	5′-GAAGGGGAGCCATTTACCCA-3′

Normalization of gene expression was performed by using the ratio of the specific gene expression/glyceraldehyde-3-phosphate dehydrogenase expression. The number of PCR cycles needed for SYBR fluorescence to cross a threshold with a significant increase in change of fluorescence (C_T = threshold cycle) was measured using the Bio-Rad iCycler software. The relative RNA expression was determined using the formula $2^{-\Delta C_T}$, where ΔC_T represents CT gene of interest−CT human glyceraldehyde-3-phosphate dehydrogenase in the experimental sample. The yield of primers was previously investigated and found to be 100%.

Membrane expression of HIV receptor (CD4) and coreceptor (CCR5) in MdM
The level of expression of CD4 and CCR5 at the cell surface of MdM was measured using flow cytometry. Untreated or treated MdM were incubated for 30 min at 4 °C with phycoerythrin-conjugated monoclonal antibodies against CD4 and CCR5 or their isotype-matched controls. Cells were then washed and fixed using Cell Fix (BD Biosciences, Le Pont de Claix, France). Viable cells were gated using forward- and side-scatter pattern. Results are expressed in molecules of equivalent fluorochrome units. Normalization of the flow cytometry analysis between donors was performed before each analysis using the calibration beads FluoroSpheres (Dakocytomation, Glostrup, Denmark).

Quantification of MIP-1α in MdM cell culture supernatants
Macrophage inflammatory protein-1α was quantified in cell culture supernatants after 7 days of treatment using a Quantikine Immunoassay kit (R&D Systems, Abingdon, UK).

Data analysis
Each experiment was performed in triplicate using human MdM obtained from one cell blood donor and repeated with MdM from two other donors. Using these three independent experiments, statistical analyses were performed using the Mann and Whitney test (Statview F 4.5, SAS Institute Inc., Cary, NC, USA).

Reagents
Cell culture reagents, macrophage-SFM and antibiotic mixtures (penicillin, streptomycin, neomycin) were purchased from Invitrogen (Lund, The Netherlands). For cell culture, macrophage-SFM was supplemented with 1% antibiotic (penicillin, streptomycin, neomycin) mixture. 5-HT, 5-HT_{1A} receptor agonist 8-OH DPAT, 5-HT_{1A} receptor antagonist WAY100635 and 5-HT_2 receptor subtype agonist αCH_3-5-HT were purchased from Sigma-Aldrich Chimie (L'isles D'abeau Chesnes, France). The p38 MAP kinase inhibitor, SB 203580, was purchased from Calbiochem (VWR International S.A.S., France). Reagents used were shown to be endotoxin-free by means of the limulus amebocyte lysate test (Chromogenix, Milano, Italy). Antibodies for flow cytometry (anti-CD4, -CD14, -CD32, -CD86, -CD163, -HLA-DR and -CCR5 and their isotype-matched controls) were purchased from BD Biosciences (San Jose, CA, USA).

Results

Effects of 5-HT on HIV replication in human MdM
The effects of 5-HT on viral replication were measured until the peak of viral replication (day 16 after infection) and results were expressed as cumulative RT activity. After 7 days

of treatment with 100 μM 5-HT, there was a significant decrease of 29% in HIV replication in untreated HIV-1-infected-MdM (Figure 1). For the 0.01 or 1 μM doses, 5-HT had no significant effect on HIV replication.

However, when 100 μM 5-HT was added during or after the HIV infection, no significant effect was evident on viral replication (Figure 1). Consequently, only pretreatment with 100 μM 5-HT was effective in decreasing HIV-1 replication in human MdM.

Effects of 5-HT, 5-HT$_{1A}$ and 5-HT$_2$ receptor agonists on HIV-1/Ba-L proviral DNA synthesis in human MdM

To define the influence of 5-HT on the early and late phases of HIV replication, the expression of proviral DNA was investigated.

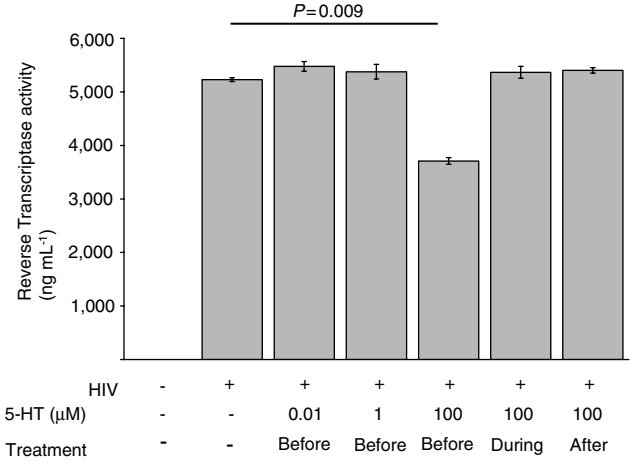

Figure 1 Effects of a long-term treatment with 5-HT (0.01, 1 and 100 μM) on HIV-1/Ba-L replication in primary cultures of human monocyte-derived macrophages. Cells were treated with 5-HT before, during or after infection with HIV-1/Ba-L at a multiplicity of infection of 1. Treatments were maintained until the peak of HIV replication was observed 16 days after infection. Results are presented as the cumulative values of reverse transcriptase (RT) activity (expressed in ng ml^{-1} of RT) until day 16 post-infection. Results are expressed as means ± s.d. of three independent experiments performed in triplicate.

With 100 μM 5-HT, a 51% decrease of HIV proviral DNA synthesis was observed: 87 ± 15 vs 178 ± 12 copies in untreated HIV-1-infected MdM ($P = 0.01$). Consistent with the previous data obtained for HIV replication, concentrations of 0.01 and 1 μM had no significant effect on HIV proviral DNA synthesis (Figure 2).

The effect of 100 μM 5-HT on HIV proviral DNA synthesis was abolished by the 5-HT$_{1A}$ receptor subtype antagonist WAY100635 (Figure 2, experimental condition no. 6). In parallel, with the 5-HT$_{1A}$ receptor agonist (8-OH DPAT), a 39% decrease of HIV proviral copies was observed (109 ± 6 vs 178 ± 12 copies in untreated HIV-1-infected MdM, $P = 0.02$; Figure 2, experimental condition no. 7). The difference in the extent of inhibition between 5-HT and 8-OH DPAT is not significant. In contrast, the 5-HT$_2$ receptor agonist (αCH_3-5-HT) had no effect on proviral DNA synthesis.

Role of the 5-HT$_{1A}$ pathway in the expression of the HIV receptor and coreceptor

To investigate a possible effect of 5-HT and the 5-HT$_{1A}$ pathway on viral entry, the levels of cell membrane expression of HIV-receptor (CD4) and coreceptor (CCR5) were evaluated using flow cytometry (Figure 3). The percentages of CD4- (Figure 3a) or CCR5- (Figure 3b) positive cells were not significantly different. Mean CD4 cell surface expression was not affected whatever the concentration of 5-HT or treatments used (10 μM WAY100635 and 50 μM 8-OH DPAT; Figure 3a). In contrast, CCR5 expression was influenced by the concentration of 5-HT (Figure 3b). We observed a dose effect of 5-HT (0.01, 1 and 100 μM) with a significant decrease of CCR5 cell surface expression in 5-HT-treated MdM (100 μM) compared with untreated MdM. This effect was abolished by the 5-HT$_{1A}$ receptor antagonist (WAY100635).

The 8-OH DPAT agonist induced a similar decrease of CCR5 expression (Figure 3b), and as for HIV DNA, no effect was observed for the 5-HT$_2$ receptor agonist αCH_3-5-HT. The cell surface expression of CCR5 was not significantly different between 5-HT-treated MdM (100 μM) and 8-OH-DPAT-treated MdM.

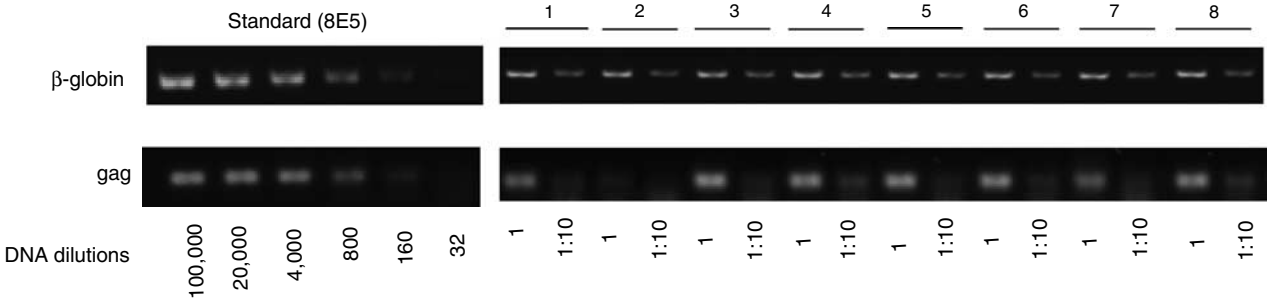

Figure 2 Effects of 5-HT and 5-HT receptor agonists on HIV proviral DNA in primary cultures of human monocyte-derived macrophages (MdM). MdM treated during 7 days with 5-HT (0.01, 1 or 100 μM), WAY100635 (10 μM), 5-HT (100 μM) + WAY100635 (10 μM), 8-OH DPAT (50 μM) or αCH_3-5-HT (50 μM) were infected with HIV-1/Ba-L (multiplicity of infection of 1) for 24 h. Two dilutions of each DNA sample were performed and the *gag* (SK38-SK39) and β-globin genes were amplified by PCR. There are eight different experimental conditions: (1) untreated MdM; (2) 5-HT-treated MdM (100 μM); (3) 5-HT-treated MdM (1 μM); (4) 5-HT-treated MdM (0.01 μM); (5) WAY100635-treated MdM (10 μM); (6) 5-HT + WAY100635-treated MdM (100 and 10 μM, respectively); (7) 8-OH-DPAT-treated MdM (50 μM) and (8) αCH_3-5-HT (50 μM). This experiment was repeated twice, with blood from two different donors, and similar results were obtained in each case.

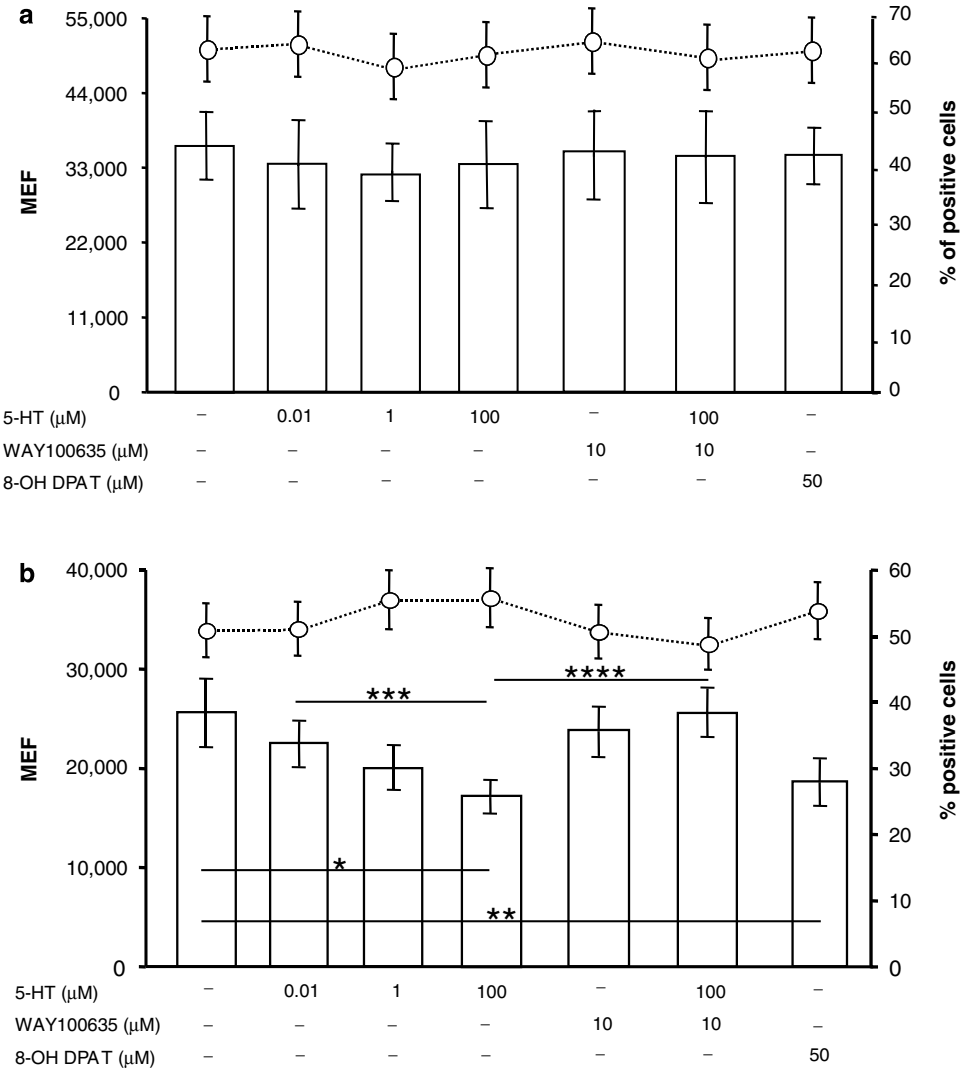

Figure 3 Effects of 5-HT on CD4 (**a**) and CCR5 (**b**) cell membrane expression in human monocyte-derived macrophages (MdM). Histograms represent the intensity of fluorescence expressed in molecules of equivalent fluorochrome (MEF). Lines with open circles represent percentages of positive cells for CD4 (**a**) and for CCR5 (**b**) biomarkers. Results are expressed as means ± s.d. for six culture wells of MdM and were generated from blood samples taken from three donors. For the expression of CCR5 at the MdM membrane, differences were considered as significant with $*P = 0.008$, $**P = 0.03$, $***P = 0.05$ and $****P = 0.007$.

Effects of 5-HT and 5-HT$_{1A}$ receptor agonist on MIP-1α secretion and mRNA expression

Treatment with 5-HT (100 μM) and the 5-HT$_{1A}$ receptor agonist (8-OH DPAT; 50 μM) induced a significant secretion of MIP-1α in MdM culture supernatants with no significant difference between 5-HT and 8-OH DPAT in terms of the MIP-1α secretion (Figure 4a). The effects of 5-HT on MIP-1α secretion were significantly abolished by the 5-HT$_{1A}$ receptor antagonist (WAY100635; 10 μM). WAY100635 had no effect on MIP-1α secretion.

Using the same concentrations of 5-HT (100 μM) and 8-OH DPAT (50 μM), MIP-1α secretion was significantly associated with a high level of MIP-1α mRNA (Figure 4b). No significant difference was observed between the effects of 5-HT and 8-OH DPAT on the MIP-1α mRNA levels.

Involvement of the p38 MAP kinase pathway on MIP-1α gene transcription induced by 5-HT was investigated (Adachi *et al.*, 2000; Richter *et al.*, 2003; Wong *et al.*, 2005). The modulation of the MIP-1α gene expression was not influenced by the p38 MAP kinase inhibitor (SB 203580) used at a concentration of 10 μM in co-treatment with 100 μM 5-HT (Figure 4b).

Discussion and conclusions

In recent studies, important roles played by 5-HT have been highlighted in terms of immune functions and the potential consequences on immunity of some drugs that target 5-HT uptake and release (O'Connell *et al.*, 2006; Evans *et al.*, 2007). This study demonstrated that, *in vitro*, 5-HT (at a pathophysiologically appropriate concentration, 100 μM) was able to decrease infection of human macrophages by HIV-1 via its action on 5-HT$_{1A}$ receptors. We found that 5-HT decreased the expression of the HIV coreceptor CCR5 and concomitantly increased MIP-1α secretion.

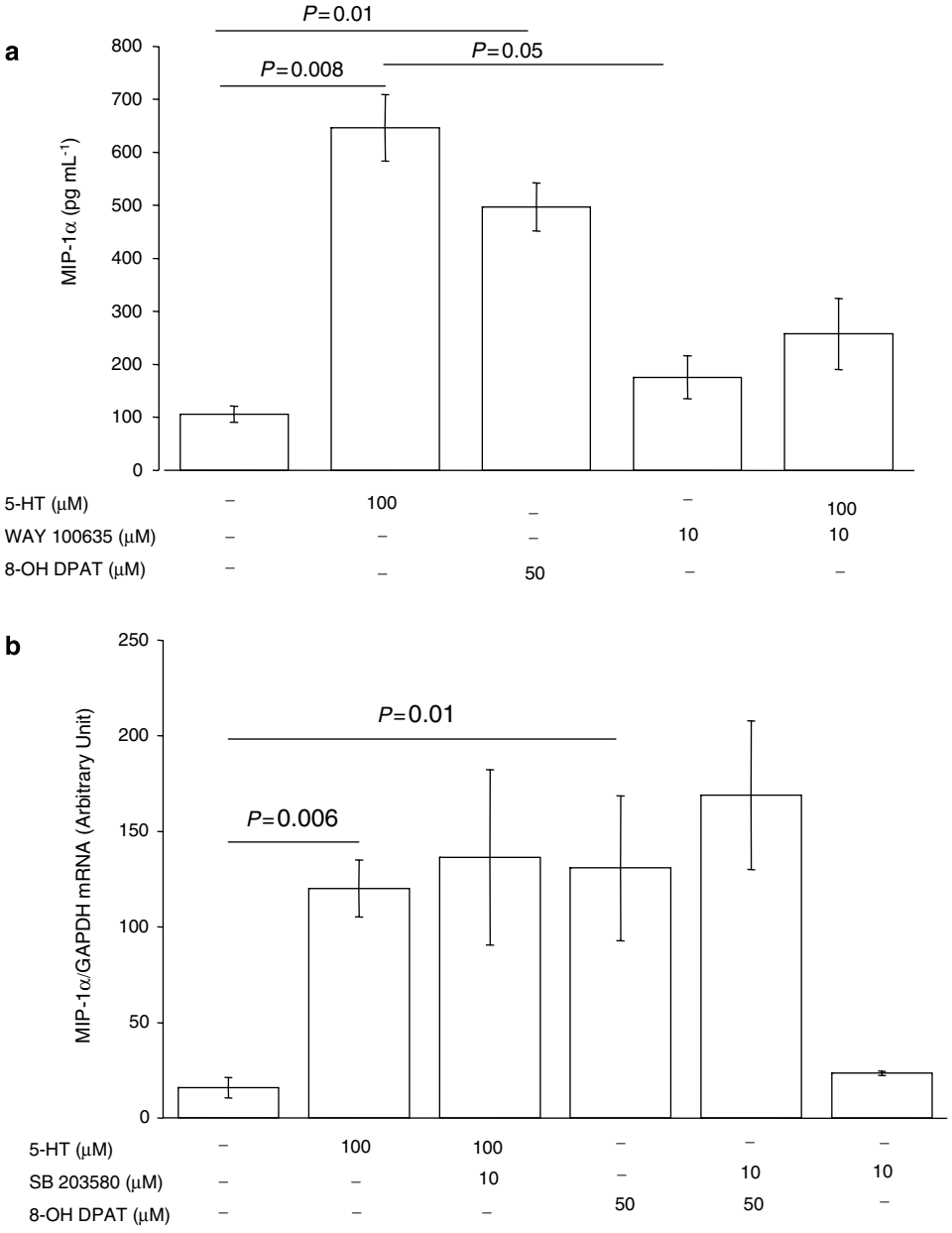

Figure 4 (**a**) Effects of 5-HT on macrophage inflammatory protein-1α (MIP-1α) secretion in primary cultures of human monocyte-derived macrophages (MdM). MIP-1α was quantified by ELISA in the supernatants of cells treated for 7 days with 5-HT (100 μM), 8-OH DPAT (50 μM), WAY100635 (10 μM) and 5-HT + WAY100635 (100 and 10 μM, respectively). (**b**) Effects of 5-HT on MIP-1α mRNA production in primary cultures of human MdM. MIP-1α mRNA was quantified by reverse transcription-PCR. Human MdM were treated during 7 days with 5-HT (100 μM), 5-HT + SB 203580 (100 and 10 μM, respectively), 8-OH DPAT (50 μM), 8-OH DPAT + SB 203580 (50 and 10 μM, respectively) or SB 203580 (10 μM) before total RNA extraction. mRNA levels were normalized on the basis of glyceraldehyde-3-phosphate dehydrogenase mRNA levels. Results, expressed as means ± s.d., are representative of those obtained with samples from three blood donors.

Leukocyte recruitment at sites of inflammation and/or infection is an early host immune response. It is clear that 5-HT is able to modulate leukocyte chemotaxis (Gordon and Barnes, 2003) and that activation and directed migration of leukocytes is partly regulated by CCR5 (Choe, 1998; Kim and Broxmeyer, 1999; Wells *et al*., 2006). To our knowledge, there is no study describing 5-HT concentrations in human tissues during the different stages of HIV infection. The highest 5-HT concentration used in this study (100 μM) is likely to be biologically relevant as it matches the levels in the micro-environment of inflammatory areas in human tissues after massive degranulation of platelets (Benedict *et al*., 1986; Mossner and Lesch, 1998).

The strong reduction of proviral DNA synthesis (51%) observed after treatment with 100 μM 5-HT was associated with only a partial decrease of HIV replication (29%). The specificity of the 5-HT effect was confirmed using an agonist of the 5-HT_{1A} receptor, 8-OH DPAT. This effect was only observed with the highest concentration of 5-HT tested (100 μM). Moreover, even considering the inhibitory effect of a 5-HT_{1A} receptor antagonist (WAY100638), it cannot be excluded that 5-HT might have activated other 5-HT receptor

subtypes. Indeed, both 5-HT_{3A} and 5-HT_4 have been found on monocytes and might be sensitive to relatively high doses of 5-HT_{1A} receptor antagonist, such as the one used in our study (Cloez-Tayarani, 2006; Cloez-Tayarani and Changeux, 2007). Moreover, although 8-OH DPAT is considered a selective agonist for the 5-HT_{1A} receptor, it can also act as a partial agonist at 5-HT_7 receptors (Bard *et al*., 1993; Shen *et al*., 1993). Recently, the presence of 5-HT_7 receptors has been shown on the surface of human T cells (Leon-Ponte *et al*., 2007), but there is no report on the presence of 5-HT_7 receptors on human macrophages. Only long-term exposures (7 days) to agonists and antagonist for 5-HT receptors have been tested. As a consequence, we cannot exclude the possibility of receptor desensitization and inactivation. Finally, the fact that only high concentration of 5-HT (100 μM) was effective may, to a certain extent, limit the extrapolation of data to pathophysiological situations. Even so, concentrations as high as 100 μM 5-HT have been detected in the context of microinflammatory areas (Benedict *et al*., 1986; Mossner and Lesch, 1998).

Interestingly, a decrease in viral infection was obtained only when 5-HT was added before but not during or after viral infection. These results imply that 5-HT acts at a very early step of the HIV infection cycle. The decrease of cell surface expression of CCR5 that was observed is likely to be associated with increased production of MIP-1α found after 5-HT or 8-OH DPAT treatments. Excess ligand could lead to increased internalization of CCR5. Moreover, MIP-1α secretion was associated with an overexpression of mRNA implying that 5-HT regulated the chemokine expression through a transcriptional mechanism. We also showed that stimulation of the 5-HT_{1A} pathway (with 8-OH DPAT) led to an increased production of MIP-1α (highest levels around 650 pg ml^{-1}). Previous studies have shown that MIP-1α concentrations of more than 100 ng ml^{-1} are able to significantly decrease HIV replication in macrophages (Capobianchi *et al*., 1998; Kelly *et al*., 1998; Cocchi *et al*., 2000; Rogez *et al*., 2003). MIP-1α is considered to be a potent inhibitor of infection of cells of the monocytic lineage by HIV-1 R5 strains (Cocchi *et al*., 1995, 1996; Alkhatib *et al*., 1996; Kelly *et al*., 1998). MIP-1α production was associated with an increase of MIP-1α mRNA synthesis, independent of the p38 MAP kinase pathway, as previously described in eosinophil cells (Adachi *et al*., 2000; Wong *et al*., 2005). This secretion of MIP-1α induced and modulated by 5-HT is likely to be involved in leukocyte recruitment and migration into inflammatory sites (Laberge *et al*., 1996; Gordon and Barnes, 2003; O'Connell *et al*., 2006).

HIV infection and replication are regulated by a complex network of immune active molecules produced by a variety of cells. It is likely that 5-HT may induce production of other host factors, such as proinflammatory cytokines, that are able to interfere with HIV-1 replication. Previous studies have found that 5-HT induced tumour necrosis factor-α and interleukin-6 production by human immune cells and that these two cytokines can significantly increase HIV-1 replication in cells of the monocytic lineage (Kubera *et al*., 2000, 2001, 2005; Cloez-Tayarani *et al*., 2003; Kedzierska *et al*., 2003). However, we did not find any modulatory effect of 5-HT on either tumour necrosis factor-α or interleukin-6 production in our human MdM culture model (data not shown).

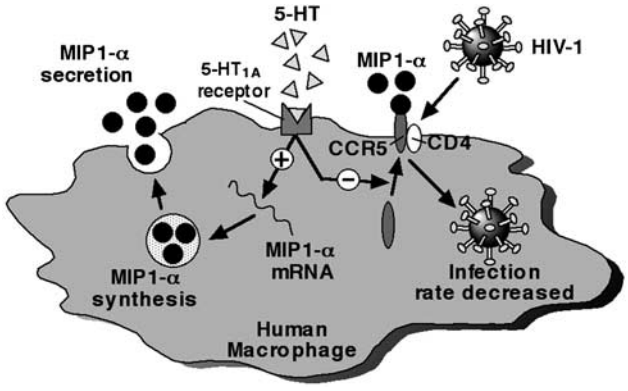

Figure 5 Scheme of effects of 5-HT on HIV-1 replication in primary cultures of human monocyte-derived macrophages (MdM), through the involvement of 5-HT_{1A} receptors. MIP-1α, macrophage inflammatory protein-1α.

Downregulation of CCR5 expression is functionally relevant to the decreased susceptibility to HIV infection. It could be either induced by CCR5 phosphorylation, as previously described in immature dendritic cells (Le *et al*., 2001), or mediated by desensitization and inactivation associated with chronic exposure to MIP-1α. The molecular mechanisms involved in the effects of 5-HT on the CCR5 pathway (and on the replication of HIV R5 strain) are unknown and require further studies. Further experiments with other specific agonists and antagonists for 5-HT_{1A} receptors and with shorter exposure time are required. Moreover, it could be of great interest to investigate the effects of 5-HT on the HIV R5X4 dual tropic strains and on the HIV X4 strains.

In conclusion, this *in vitro* study suggests that 5-HT can reduce HIV-1 entry in human macrophages by two mechanisms, downregulation of CCR5 and/or increase of MIP-1α secretion. This study showed that 5-HT may decrease the availability of the main HIV coreceptor (CCR5) and concomitantly increase the concentration of the natural ligand for CCR5, MIP-1α, thereby making the receptor even less accessible to the virus (Figure 5).

The functional role of 5-HT in the immune system has received increasing attention (Mossner and Lesch, 1998; Mizruchin *et al*., 1999; Dustin and Colman, 2002; Evans *et al*., 2007). These data provide new evidence for the immune properties of 5-HT in the complex interactions between the immune system and the CNS, and their possible involvement in the control of viral dissemination in brain.

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Conflict of interest

The authors state no conflict of interest.

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