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Modulation of the proteome of peripheral blood mononuclear cells from HIV-1 infected patients by drugs of abuse

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Abstract

We used proteomic analyses to assess how drug abuse modulates immunologic responses to infections with the human immunodeficiency virus type 1 (HIV-1). Two dimensional (2D) difference gel electrophoresis was utilized to determine changes in the proteome of peripheral blood mononuclear cells (PBMC) isolated from HIV-1 positive donors that occurred after treatment with cocaine or methamphetamine. Both drugs differentially regulated the expression of several functional classes of proteins. We further isolated specific subpopulations of PBMC to determine which subpopulations were selectively affected by treatment with drugs of abuse. Monocytes, B cells and T cells were positively or negatively selected from PBMC isolated from HIV-1 positive donors. Our results demonstrate that cocaine and methamphetamine modulate gene expression primarily in monocytes and T cells, the primary targets of HIV-1 infection. Proteomic data were validated with quantitative, real-time PCR. These studies elucidate the molecular mechanisms underlying the effects of drugs of abuse on HIV-1 infections. Several functionally relevant classes of proteins were identified as potential mediators of HIV-1 pathogenesis and disease progression associated with drug abuse.

Keywords

cocaine; methamphetamine; peripheral blood mononuclear cells (PBMC); human immunodeficiency virus type 1 (HIV-1); difference gel electrophoresis (DIGE); high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS)

Introduction

The human immunodeficiency virus type 1 (HIV-1), a retrovirus, causes the acquired immunodeficiency syndrome (AIDS) which results in immune system collapse rendering the infected patient susceptible to life threatening opportunistic infections and malignancies. Approximately 33.2 million people worldwide are living with HIV-1/AIDS, and 2.5 million people are estimated to have contracted HIV-1 in 2007 (1). In the US, an estimated 1.1 million people are living with HIV-1/AIDS (2). Transmission of HIV-1 generally occurs through infected body fluids (sexual transmission) or parenteral transmission (intravenous drug abusers) (3).

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Cocaine and methamphetamine are widely abused drugs, both of which can be snorted, smoked or injected (3). In 2007, the number of new users of cocaine and methamphetamine in the US among persons aged 12 years or older was 906,000 and 157,000, respectively (1–3). From the beginning of the HIV/AIDS epidemic through 2005, an estimated 225,210 cases are believed to be transmitted through injection drug use (1,5). Current literature suggests that cocaine or methamphetamine usage may exacerbate the immunopathogenesis of HIV-1 (5–8). Cocaine and methamphetamine have both been shown to enhance HIV-1 replication in immune cells including macrophages, dendritic cells and peripheral blood mononuclear cells (PBMC) (9–13). Drug abuse also has been associated with the development of strains of drug resistant HIV-1 (5,14,15). Ellis et al. (14) demonstrated that HIV-1 positive methamphetamine users who were receiving highly active anti-retroviral therapy (HAART) had higher viral loads than HIV-1 positive methamphetamine users who were not receiving therapy. Additionally, a dual tropic, multidrug resistant HIV-1 strain was isolated from a methamphetamine user (5). This patient had rapid HIV-1 seroconversion with progression to symptomatic AIDS in 4–20 months. Moreover, antiretroviral naïve patients who use methamphetamine are more likely to have drug resistance to non-nucleoside reverse transcriptase inhibitors than methamphetamine non-users (15).

Understanding how cocaine or methamphetamine modulates the immune response to HIV-1 has not been fully explored. In this paper, we test the hypothesis that cocaine or methamphetamine regulates the expression of various host proteins in PBMC isolated from HIV-1 positive donors. These proteins may directly or indirectly play a role in increased progression of HIV-1 infection. We used 2 Dimensional (2D) difference gel electrophoresis (DIGE) to elucidate the proteomic changes that occurred when PBMC isolated from HIV-1 positive donors were treated with cocaine or methamphetamine. We validated our proteomic results using quantitative real-time PCR. We further investigated which subpopulations of cells were selectively affected by drugs of abuse. Monocytes, B cells and T cells were positively or negatively selected from PBMC isolated from HIV-1 positive donors. Our results demonstrate that cocaine and methamphetamine modulate gene expression primarily in monocytes and T cells.

METHODS

Human Subjects

Blood donors were apprised of this study and consents were obtained consistent with the policies of the appropriate local institutions and the National Institutes of Health. Peripheral blood samples from HIV-1 positive patients were drawn into a syringe containing heparin (20 units/ml). The HIV-1 positive patients were recruited from the Immunodeficiency Services Clinic of the affiliated Erie County (NY) Medical Center.

Isolation of human PBMC

PBMC were isolated by density gradient centrifugation on Ficoll-Paque (Amersham Pharmacia Biotech, Piscataway, NJ). *Isolation of PMBC subpopulations:* Subpopulations of PBMC were separated using Dynabeads (Invitrogen) according to the manufacturer's instructions for positive isolation of monocytes (Dynabeads[®] CD14), positive isolation of B cells (Dynabeads[®] CD19 Pan B; DETACHaBEAD[®] CD19), and negative isolation of T cells (Dynabeads[®] Untouched[™] Human T Cells). Cells were cultured in RPMI 1640 medium containing 25 mM HEPES buffer supplemented with 10% heat-inactivated fetal calf serum (Invitrogen, Grand Island, NY), 80 mg of gentamicin/ml (Sigma-Aldrich, St. Louis, MO) and 300 mg/ml glutamine (complete medium).

Drug Treatment

PBMC, monocytes, B cells and T cells isolated from HIV-1 positive donors were treated with cocaine (Sigma-Aldrich) at 1 μ M for 48 hr or methamphetamine hydrochloride (Sigma-Aldrich) at 100 μ M for 24 hr. Control cells were treated with complete media alone. The concentrations of cocaine and methamphetamine used were based on previous dose response studies that produced a maximum biological response without causing toxicity to the target cells and also were based on published studies (11–13). The concentrations of cocaine and methamphetamine are comparable to levels found in the blood, urine or tissue samples of users which range from ≤ 1 μ M to 600 μ M (16–21).

2D DIGE

The Ettan DIGE technique (GE Healthcare) was used to detect differences in protein abundance between normal and experimental samples. The Ettan DIGE system uses three CyDye DIGE fluors (Cy2, Cy3, Cy5), each with a unique fluorescent wavelength, matched for mass and charge. CyDyes form a covalent bond with the free epsilon amino group on lysine residues from the sample proteins. CyDyes label approximately 2% of the lysine residues. This system allows for two experimental samples and an internal standard to be simultaneously separated on the same gel. The internal standard is comprised a pool of an equal amount of all the experimental samples. The use of an internal standard facilitates accurate inter-gel matching of spots, and allows for data normalization between gels to minimize gel to gel experimental variability (22).

Sample preparation

After stimulation, cells were washed 2 times with 1X PBS (Invitrogen, Grand Island, NY). Total protein was extracted using standard cell lysis buffer [30 mM TrisCl; 8 M Urea; 4% (w/v) 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), adjusted to pH 8.5] for 10 min on ice. The cell lysate was centrifuged at 4°C for 10 min at 12000 g and the lysate was further purified by precipitation with chloroform/methanol as described (23). Cell lysates were resuspended in standard cell lysis buffer. Final cell lysate protein concentrations were determined with Bio-Rad Coomassie Protein Reagent (Bio-Rad, Hercules, CA) and used for DIGE analysis.

Sample labeling

All reagents used were from GE Healthcare. Briefly, 50 μ g of cell lysate was labeled with 400 pmol of either Cy3 or Cy5 or Cy2 (Cy 2 was used to label the internal standard) on ice for 30 min and then quenched with a 50-fold molar excess of free lysine. Cy3, Cy5, Cy2 labeled samples and unlabeled protein (500–800 μ g) were combined. An equal volume of 2X sample buffer [8 M Urea; 2% (v/v) Pharmalytes 3-10; 2% (w/v) dithiothreitol (DTT); 4% (w/v) CHAPS] was added and incubated on ice for 10 min. The total volume of sample was adjusted to 450 μ l with rehydration buffer [4% (w/v) CHAPS; 8 M Urea; 1% (v/v) Pharmalytes 3-10 nonlinear (NL); 13 mM DTT].

DIGE

Samples were applied to immobilized pH gradient (IPG) strips (24cm, pH 3-10 NL), and absorbed by active rehydration at 30 V for 13 hr. Isoelectric focusing was carried out using an IPGphor IEF system with a three phase program; first phase at 500 V for 1 hr, second phase at 1000 V for 1 hr, and third phase (linear gradient) 8000 to 64000 V for 2 hr (50 μ A maximum per strip). Prior to separation in the second dimension, strips were equilibrated for 15 min in equilibration buffer I [50 mM Tris-HCl, 6 M Urea, 30% (v/v) glycerol, 2% (w/v) SDS, 0.5% (w/v) DTT]. The strips were again equilibrated for 15 min in equilibration buffer II [50 mM Tris-HCl, 6 M Urea, 30% (v/v) glycerol, 2% (w/v) SDS, 4.5% (w/v) iodoacetamide] and

transferred onto 18×20 cm, 12.5% uniform polyacrylamide gels poured between low fluorescence glass plates. Gels were bonded to inner plates using Bind-Silane solution according to the manufacturer's protocol. Strips were overlaid with 0.9% agarose in 1X running buffer containing bromophenol blue and were run for 16 hr (1.8 W per gel) overnight at 15° C, in the Ettan DALT electrophoresis system.

Image acquisition

Fluorescent images of each CyDye (Cy3, Cy5, Cy2) were acquired using a Typhoon 9410 variable mode imager. After scanning, gels were fixed in 30% (v/v) methanol, 7.5% (v/v) acetic acid for 3 hr and stained overnight at room temperature with SYPRO-Ruby dye (Molecular Probes, Eugene, OR). Gels were de-stained in water and then scanned using the Typhoon 9410 scanner.

Image analysis

Images were processed in ImageQuant v5.2 software (GE Healthcare), and imported into DeCyder differential in-gel analysis (DIA) software v5.0 (GE Healthcare) for spot detection and normalization of spot intensities within each gel. Intergel matching was performed using the biological variation analysis (BVA) component of DeCyder software for cross-gel statistical analysis. Standardized protein abundance was calculated by dividing each Cy3 or Cy5 spot volume by the corresponding Cy2 standard spot volume (internal control) within each gel, and the difference in standardized abundance between control and drug-treated PBMC was expressed as the average volume ratio (data represented as % change from control). Statistical analysis (Student's *t*-test) was performed on spots matched across gels. Protein spots that showed statically significant differences between the control and drug-treated samples were picked using the Ettan Spot Picker (22) for subsequent mass spectrometry.

High performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS)

In gel digestion of excised spots and HPLC-MS/MS were performed as described by Breci et al. 2005 (24). Briefly, gel slices were destained (25) and digested with trypsin (26). The tryptic peptides were extracted with 5% formic acid/50% CH₃CN. HPLC was performed using a microbore system (Surveyor, ThermoFinnigan, San Jose, CA). The HPLC column eluate was directed into a ThermoFinnigan LCQ Deca XP Plus ion trap mass spectrometer. Automated peak recognition, dynamic exclusion, and daughter ion scanning of the top two most intense ions were performed using Xcalibur software (27). Spectra were scanned over the range 400–1400 mass units. MS/MS data were analyzed using SEQUEST, a computer program that allows the correlation of experimental data with theoretical spectra generated from known protein sequences. A preliminary positive peptide identification for a doubly-charged peptide was based upon a correlation factor (Xcorr) greater than 2.5, a delta cross-correlation factor (dCn) greater than 0.1 (indicating a significant difference between the best match reported and the next best match), a high preliminary scoring, and a minimum of one tryptic peptide terminus. For triply-charged peptides the correlation factor threshold was set at 3.5. All matched peptides were confirmed by visual examination of the spectra, and all spectra were searched against the latest version of the public, non-redundant protein database of the National Center for Biotechnology Information (NCBI) (28,29).

RNA extraction and real time, quantitative PCR (Q-PCR)

Cytoplasmic RNA was extracted using TRIzol from Invitrogen (30). The final RNA pellet was dried and resuspended in diethyl pyrocarbonate (DEPC) water and the concentration of RNA was determined using a spectrophotometer at 260 nm. Any DNA contamination in the RNA preparation was removed by treating the RNA with DNase (1 IU/μg of RNA, Promega, Madison WI) for 2 hr at 37°C, followed by proteinase K digestion at 37°C for 15 min and

subsequent extraction with phenol/chloroform and $\text{NH}_4\text{OAc}/\text{EtOH}$ precipitation. The isolated RNA was stored at -70°C until used. DNA contamination of the RNA preparation was checked by including a control in which reverse transcriptase enzyme was not added to the PCR amplification procedure. Relative abundance of each mRNA species was assessed using the SYBR green master mix from Stratagene (La Jolla, CA) to perform Q-PCR. Differences in threshold cycle number were used to quantify the relative amount of PCR target contained within each tube. Relative mRNA species expression was quantitated and expressed as transcript accumulation index ($\text{TAI} = 2^{-(\Delta\Delta\text{CT})}$), calculated using the comparative C_T method. All data were controlled for quantity of RNA input by performing measurements on an endogenous reference gene, β -actin (31).

Statistics

For gene expression data, statistical significance was determined using a Student's t-test (SPSS Inc.).

Results

Cocaine differentially modulates the expression of proteins in PBMC isolated from HIV-1 positive donors

Figure 1 is a representative 2D gel image of SYPRO Ruby dye stained proteins. Comparing standardized protein abundance data generated from the Cy3, Cy5 and Cy2 images (DeCYDER software) several protein spots were differentially expressed between cocaine treated and untreated, control PBMC. These protein spots were excised from the SYPRO Ruby stained gel and identified by HPLC-MS/MS. The protein spots that yielded positive identification are shown in Figure 1; they are labeled with arbitrary spot numbers and their identities are listed in Table I. Proteins that had a significant increase in expression were poly(rC) binding protein 1, vimentin, triosephosphate isomerase 1, chromobox homolog 3, sorcin, and galectin-1. Proteins that had a significant decrease in expression were proteasome $\alpha 1$ subunit, heat shock 70kDa protein 5 (HSP70p5), β globin, tubulin, rho GDP dissociation inhibitor- α (rho GDI α), rho GDP dissociation inhibitor- β (rho GDI β), tumor protein D54, proteasome $\alpha 3$ subunit, calpain, tyrosine 3-monooxygenase-tryptophan 5-monooxygenase activation protein, peroxiredoxin 6, and talin 1.

Methamphetamine differentially modulates the expression of proteins in PBMC isolated from HIV-1 positive donors

Figure 2 is a representative 2D gel image of SYPRO Ruby dye stained proteins. Comparing standardized protein abundance data generated from the Cy3, Cy5 and Cy2 images several proteins spots were differentially expressed between methamphetamine treated and untreated control PBMC cultures isolated from HIV-1 positive donors. These protein spots were excised from the SYPRO Ruby stained gel and identified by HPLC-MS/MS and are shown in Figure 2, labeled with arbitrary spot numbers. Protein identities are listed in Table II. The identified spots that had increased protein expression were: vimentin, lactate dehydrogenase, calponin 2, fibrinogen gamma chain, RNA-binding protein regulatory subunit, transgelin 2, poly(rC) binding protein 1, proteasome $\alpha 3$ subunit, and galectin-1. The identified spots that had decreased protein expression were: tubulin, HSP70p5, glutathione-S-transferase, adenine phosphoribosyltransferase, erythrocyte band 7 integral membrane protein, superoxide dismutase, and peroxiredoxin 6.

For all 2D gels, several weak protein spots could not be identified due to insufficient amounts for identification and therefore are not shown in Figure 1 and Figure 2.

Modulation of gene expression

To confirm our proteomics data and demonstrate that gene expression correlates with protein synthesis, we investigated the effect of cocaine or methamphetamine on the expression of mRNA levels of specific genes by Q-PCR analysis using the primers listed in Table III. *Effects of cocaine:* Figure 3 shows that cocaine treatment had no effect on 18s RNA expression used as control RNA. Furthermore, cocaine significantly increased gene expression of vimentin, poly(rc) binding protein, and galectin-1 in PBMC isolated from HIV-1 positive donors while significantly decreasing gene expression of HSP70p5, rho GDI α , rho GDI β and peroxiredoxin 6. Cocaine induced changes in gene expression are reflective of changes in protein expression. *Effects of methamphetamine:* Figure 4 show that methamphetamine treatment had no effect on 18s RNA expression used as control RNA. Methamphetamine significantly increased gene expression for vimentin, poly(rc) binding protein, and galectin-1 while significantly decreasing gene expression for HSP70p5, glutathione-S-transferase, superoxide dismutase, and peroxiredoxin 6 in PBMC isolated from HIV-1 positive donors.

Modulation of gene expression in PBMC subpopulations

To define the specific cell populations whose gene expression was selectively modulated by drugs of abuse, monocytes, B cells and T cells were isolated from PBMC obtained from HIV-1 positive donors. Cell subpopulations were subsequently treated with cocaine (1 μ M, 48 hr) or methamphetamine (100 μ M, 24 hr) and the expression of mRNA levels of the genes in PBMC previously shown to be modulated by cocaine and methamphetamine was investigated by Q-PCR analysis. *Effects of cocaine:* In both monocytes and T cells (Figure 5a & 5b) cocaine significantly increased gene expression of vimentin, poly(rc) binding protein, and galectin-1 while significantly decreasing gene expression of HSP70p5 and peroxiredoxin 6. Cocaine had no significant effect on gene expression in B cells (data not shown). *Effects of methamphetamine:* Methamphetamine significantly increased gene expression of vimentin, poly(rc) binding protein, and galectin-1 in both monocytes and T cells (Figure 6a & 6b). Furthermore, a significant decrease in gene expression of HSP70p5 and peroxiredoxin 6 was observed in monocytes and T cells from HIV-1 positive donors (Figure 6a & 6b). Methamphetamine had no significant effect on gene expression in B cells (data not shown). Therefore, the effects of drugs of abuse are primarily on monocytes and T cells, the primary targets of HIV-1, from HIV-1 positive donors.

Discussion

Drug abuse has long been recognized as a significant co-morbidity associated with HIV-1 infections. In this study we sought to gain insight into the molecular mechanisms underlying the effects of drug abuse on progression of HIV-1 infections. Specifically, we examined changes in the proteome of PBMC from HIV-1 infected donors in response to treatment with the widely used, addictive drugs, cocaine and methamphetamine. Our data show that several functionally important classes of proteins were differentially regulated. The potential relevance of the differential expression of select regulated proteins is discussed below.

Heat shock proteins (HSPs), also known as stress proteins, belong to the family of molecular chaperones that can be induced upon cellular injury. HSPs are classified by their molecular weight, comprising of six general families: HSP110, HSP90, HSP70, HSP60, small molecular weight Hsps, and immunophilins (32,33). HSPs have diverse functions including chaperone activity, regulation of redox state and regulation of protein turnover (32–34). HSP70 is incorporated into the membrane of HIV-1, HIV-2 and simian immunodeficiency virus (SIV) virions (35) and may affect the nuclear import of the HIV-1 preintegration complex (36). Overexpression of HSP70 decreases HIV-1 Vpr protein induced G₂ arrest and apoptosis in 293T-632 cells which could be reversed with HSP70 RNA interference (37). These studies

suggest that HSP70 is a novel, anti-HIV-1 innate immunity factor that targets HIV-1 Vpr (37,38). This study demonstrates that cocaine and methamphetamine both decrease the expression of heat shock 70kDa protein 5 in PBMC, monocytes and T cells isolated from HIV-1 positive donors thereby supporting the premise that cocaine and methamphetamine suppress this innate immunity factor.

Vimentin is an intermediate filament protein that plays a role in mechanical and biological functions such as cell contractility, migration, stiffening, and proliferation (39). HIV-1 utilizes the host cytoskeletal system for infection and replication. HIV-1 and HIV-2 proteases have been shown to cleave vimentin (40–43). Furthermore, cleavage of vimentin by HIV-1 proteases is necessary for changes in chromatin organization and distribution induced by HIV-1 (44). In HeLa cells, the HIV-1 viral infectivity factor (Vif) colocalizes with vimentin in perinuclear aggregates (45,46). Additionally, vimentin expression was shown to be decreased in SVGA-Tat expressing cells (47). Both cocaine and methamphetamine increase the expression of vimentin in PBMC, monocytes and T cells isolated from HIV-1 positive donors. An increase in expression of vimentin may play a role in facilitating the spread of HIV-1 infection to neighboring cells.

Cocaine and methamphetamine both down-regulate the antioxidant protein, peroxiredoxin 6. Previous studies have shown that peroxiredoxins are capable of inhibiting HIV-1 infection and members of the peroxiredoxin family, NKEF-A or NKEF-B inhibit HIV-1 replication in CD8⁺ T cells (48). Our studies support the premise that drugs of abuse increase susceptibility to and progression of HIV-1 infections by down-regulating the expression of the antioxidant protein, peroxiredoxin 6, which also has anti-HIV-1 activities.

We also found that members of the family of small GTP-binding proteins, RhoGDI alpha and RhoGDI beta, were down-regulated by cocaine treatment of PBMC isolated from HIV-1 positive patients. These proteins stabilize the inactive GDP-bound form of GTPases, preventing the release of GDP and the binding of GTP (49). GTPases are necessary for HIV-1 replication (50–52), therefore our results indicate that cocaine-induced down-regulation of RhoGDI synthesis may enhance viral replication by allowing continual activation of GTPases.

Antioxidants such as superoxide dismutase are an important defense for cells. Superoxide dismutase converts superoxide to hydrogen peroxide, preventing the formation of reactive oxygen species. Studies demonstrate that a high activity of endogenous antioxidants may have a protective role on CD4⁺ T-cells, which may limit HIV-1 infection (53). Furthermore, cultured mature neurons transduced with rSV40 vectors carrying human SOD1 were protected against HIV-1 gp120 induced apoptosis. (54). This study demonstrates that methamphetamine decreases the production of superoxide dismutase. These data and previous studies suggest that methamphetamine treatment reduces the ability of PBMC to inhibit HIV-1 infection.

Galectin-1 is a member of a family of lectins with affinity for β -galactosidase that are defined by a shared amino acid sequence in their carbohydrate recognizing domain. Galectin-1 is widely expressed in the tissues of mammals including the lung, brain, heart, spleen and lymph nodes (55). Galectin-1 is located both extracellularly and intracellularly. Galectin-1 modulates cell proliferation, apoptosis, cell cycle arrest, cell-matrix adhesion, and cell to cell adhesion (55,56). Previous studies demonstrated that galectin-1 acts as a soluble HIV-1 binding protein that can stabilize virus-cell interactions and promote virus replication in PBMC, CD4⁺ T cells, and monocyte derived macrophages (57,58). Our data demonstrate that both cocaine and methamphetamine up-regulate the expression of galectin-1 in PBMC, monocytes and T cells isolated from HIV-1 positive donors, thereby enhancing HIV-1 infection by providing a mechanism for virus-to-cell interactions.

Conclusion

Our results show that cocaine and methamphetamine differentially regulate the synthesis of several specific proteins in PBMC, monocytes and T cells isolated from HIV-1 positive donors. However, the functions of these proteins with respect to HIV-1 disease progression remain to be clearly delineated. Many of these proteins have been reported to be involved in the pathogenesis of HIV-1 infections and may eventually serve as either biomarkers of disease progression or as unique targets for therapy in drug abusing, HIV-1 infected patients.

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Figure 1.

Two-dimensional gel electrophoresis of cocaine-induced, differentially expressed proteins in PBMC isolated from HIV-1 positive donors. This is a representative 2D SYPRO-Ruby stained gel image ($n = 4$ independent experiments). Arbitrary spot numbers indicate statistically significant (Student's t -test), differentially expressed proteins. The pH increases from left to right and the molecular mass decreases from the top to the bottom of the gels.

Figure 2.

Two-dimensional analyses of methamphetamine-induced, differentially expressed proteins in PBMC isolated from HIV-1 positive donors. This is a representative 2D SYPRO-Ruby stained gel image ($n = 4$ independent experiments). Arbitrary spot numbers indicate statistically significant (Student's t -test), differentially expressed proteins. The pH increases from left to right and the molecular mass decreases from the top to the bottom of the gels.

Figure 3.

Cocaine-induced changes in gene expression measured by Q-PCR in PBMC isolated from HIV-1 positive donors. Data are presented as the mean $\pm$ SD of 4 independent experiments; * $p \leq 0.001$, # $p \leq 0.01$

Figure 4. Methamphetamine-induced changes in gene expression measured by Q-PCR in PBMC isolated from HIV-1 positive donors. Data are presented as the mean $\pm$ SD of 4 independent experiments; * $p \leq 0.001$, # $p \leq 0.01$

Figure 5.

(a) Cocaine-induced changes in gene expression measured by Q-PCR in monocytes isolated from HIV-1 positive donors. Data are presented as the mean $\pm$ SD of 4 independent experiments; * $p \leq 0.001$, # $p \leq 0.01$. (b) Cocaine-induced changes in gene expression measured by Q-PCR in T cells isolated from HIV-1 positive donors. Data are presented as the mean $\pm$ SD of 4 independent experiments; * $p \leq 0.001$, # $p \leq 0.01$

Figure 6.

(a) Methamphetamine-induced changes in gene expression measured by Q-PCR in monocytes isolated from HIV-1 positive donors. Data are presented as the mean $\pm$ SD of 4 independent experiments; * $p \leq 0.001$, # $p \leq 0.01$. (b) Methamphetamine-induced changes in gene expression measured by Q-PCR in T cells isolated from HIV-1 positive donors. Data are presented as the mean $\pm$ SD of 4 independent experiments; * $p \leq 0.001$, # $p \leq 0.01$.

Table 1

PBMC isolated from HIV-1 positive subjects were cultured with and without cocaine (1 μ M) for 48 hr (n = 4 independent experiments). Data represent statistically significant differentially expressed proteins (Student's t-test) that were identified using HPLC-MS/MS (all proteins had an expect score >10). Data are represented as protein name, gene accession number (Gi No.), theoretical mass, theoretical isoelectric point (pI), ratio and significance. Theoretical mass and pI were calculated using: http://au.expasy.org/tools/pi_tool.html.

Spot Number	Protein name	Accession Number	Theoretical mass	Theoretical pI	ratio	p value
1623	poly(rC) binding protein 1	gi 6754994	37497.81	6.66	1.78	0.002
1812	vimentin	gi 2119204	53651.68	5.06	1.53	0.001
2397	triosephosphate isomerase 1	gi 4507645	26669.49	6.45	1.59	0.005
2596	chromobox homolog 3	gi 6680860	19752.13	4.96	1.38	0.01
2653	sorcin	gi 4507207	21676.38	5.32	1.42	0.002
2653	galectin 1	gi 4504981	14715.70	5.33	1.42	0.002
1543	integrin α -IIb	gi 124951	113390.90	5.21	-1.35	0.001
1773	proteasome α 1 subunit	gi 23110935	30239.44	6.51	-1.22	0.003
1596	heat shock 70kDa protein 5 (HSP70 p5)	gi 16507237	72332.96	5.07	-1.41	0.05
2058	β globin	gi 4504349	15998.41	6.74	-1.64	0.042
2051	tubulin	gi 20903441	50151.63	4.94	-1.20	0.035
2282	rho GDP dissociation inhibitor- α (rho GDI α)	gi 4757768	23207.11	5.03	-1.35	0.001
2282	Tumor protein D54	gi 20141658	22237.73	5.26	-1.35	0.001
2287	rho GDP dissociation inhibitor- β (rho GDI β)	gi 10835002	22988.01	5.10	-1.31	0.006
2308	proteasome α 3 subunit	gi 4506183	28433.23	5.19	-1.41	0.002
2385	calpain	gi 4502565	28315.71	5.05	-1.41	0.002
2385	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	gi 4507949	28082.40	4.76	-1.41	0.002
2408	peroxiredoxin 6	gi 4758638	25034.99	6.00	-1.62	0.035
2408	talin 1	gi 14916725	269717.94	5.72	-1.62	0.035

Table II

PBMC isolated from HIV-1 positive subjects were cultured with and without methamphetamine (100 µm) for 24 hr. Data represent statistically significant differentially expressed proteins (Student's t-test) that were identified using HPLC-MS/MS (all proteins had an expect score >10). Data are represented as protein name, gene accession number (Gi No.), theoretical mass, theoretical isoelectric point (pI), ratio and significance. Theoretical mass and pI were calculated using: http://au.expasy.org/tools/pi_tool.html.

Spot #	Protein Name	Gi	Theoretical mass	Theoretical pI	ratio	p value
1108	vimentin	gi 62414289	53651.68	5.06	1.59	0.033
1586	lactate dehydrogenase	gi 32693754	36557.53	8.46	1.53	0.026
1597	calponin 2	gi 4758018	33697.05	6.94	2.18	0.05
1737	fibrinogen gamma chain	gi 182439	44707.05	6.56	1.72	0.05
2068	RNA-binding protein regulatory subunit	gi 14198257	19847.01	6.33	1.78	0.007
2035	transgelin 2	gi 4507357	22391.45	8.41	1.59	0.025
1309	poly(rC) binding protein 1	gi 6754994	37497.81	6.66	1.64	0.073
1735	proteasome α 3 subunit	gi 23110935	30239.44	6.51	1.63	0.041
2053	galectin 1	gi 4504981	14715.70	5.33	1.57	0.001
1726	tubulin	gi 20903441	50151.63	4.94	-1.87	0.05
1732	heat shock 70kDa protein 5 (HSP70 p5)	gi 16507237	72332.96	5.07	-1.50	0.05
1773	glutathione-S-transferase	gi 4758484	27565.86	6.24	-1.36	0.05
2047	adenine phosphoribosyltransferase	gi 4502171	19607.77	5.78	-1.47	0.01
2047	erythrocyte band 7 integral membrane protein	gi 114823	31730.80	7.71	-1.47	0.01
2050	superoxide dismutase	gi 34707	22089.00	6.86	-2.16	0.027
2050	peroxiredoxin 6	gi 4758638	25034.99	6.00	-2.16	0.035

Table III

Primer sequences for Real Time Q-PCR

Primer	Primer sequences
β -actin	5', 5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3' 3', 5-AGTCATAGTCCGCCTAGAAGCATTGCGGT-3'
galectin-1	5' 5'-CTC TCG GGT GGA GTC TTC TG-3' 3', 5-GAA GGC ACT CTC CAG GTT TG-3'
glutathione-S-transferase	5', 5'-AAG GCC CTG AAA ACC AGA AT-3' 3' 5'-GCC TCC ATG ACT GCG TTA TT-3'
HSP70 p5	5' 5'-TAG CGT ATG GTG CTG CTG TC-3' 3' 5'-TTT GTC AGG GGT CTT TCA CC-3'
peroxiredoxin 6	5', 5'- GGA TGG GGA TAG TGT GAT GG-3' 3', 5'- CTG ACA TCC TCT GGC TCA CA-3'
poly(rC) binding protein	5', 5'-GGA AGC ATC ATT GGG AAG AA-3' 3', 5'-TCT TCC TCC AGC TTG TCG AT-3'
rho GDI α	5', 5'- GAG CCT GCG AAA GTA CAA GG-3' 3', 5'- TCC TTC AGC ACA AAC GAC TG-3'
rho GDI β	5', 5'- CCT ACA GGA CTG GGG TGA AA-3' 3', 5'- GAG CCT CCT CAA CTG GAG TG-3'
superoxide dismutase	5', 5'-AGG GCA TCA TCA ATT TCG AG-3' 3' 5'-ACA TTG CCC AAG TCT CCA AC-3'
vimentin	5', 5'-GAG AAC TTT GCC GTT GAA GC-3' 3' 5'-TCC AGC AGC TTC CTG TAG GT-3'
18S	Purchased from Ambion (Austin, TX)