HIV-Tat and Cocaine Impact Brain Energy Metabolism: Redox Modification and Mitochondrial Biogenesis Influence NRF-Transcriptional Mediated Neurodegeneration

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Abstract

HIV infection and drugs of abuse induce oxidative stress and redox imbalance, which cause neurodegeneration. The mechanisms by which HIV infection and cocaine consumption affect astrocytes energy metabolism lead neurodegenerative dysfunction remain poorly understood. We investigated how oxidative injury cause energy resource and depletion of glutathione synthetase (GSS), activating energy sensor AMPK-mediated glycolytic enzymes, and mitochondrial biogenesis lead to NRF transcription factors in astrocytes. Both human primary astrocytes *in vitro* (HIV-1Tat/cocaine) and HIV-inducible Tat (iTat) mice exposed to cocaine inhibit the GSS and altered superoxide dismutase (SOD) level. This, in turn, significantly activated AMPK and raised the several glycolytic enzymes, oxidative phosphorylation, mitochondrial biogenesis of PGC-1α and TFAM, Nrf1 and Nrf2 gene transcription, and protein expression. These results suggest that redox inhibition of GSS altered AMPK activation and mitochondrial biogenesis influence Nrf transcriptions, which are important resource of astrocytes signaling network in brain energy metabolism in HIV-positive cocaine users. In conclusion, HIV-1 Tat altered redox inhibition, thus raising glycolytic metabolic profiles and mitochondrial biogenesis led to Nrf transcription, which impact astrocyte-energy resource and metabolism. Further cocaine comorbidity exacerbation of these effects led to worse in neurodegeneration.

Introduction

Human immunodeficiency virus (HIV) is one of the major causes of human death. HIV infection either directly or indirectly affects the central nervous system (CNS), causing neurological impairments like HIV-associated neurocognitive disorders (HAND), which are manifested by a massive death of neurons in brain regions [1]. HIV-1 infection primarily affects immune cells like microglial cells and subsequently impacts astrocytes and neurons [2]. The HIV-infected cells and activated immune cells in the brain release viral proteins like the HIV-1 Transactivator protein (Tat). HIV-1 Tat is a 14–16 kDa protein and a key activator of HIV transcription in host cells. HIV-1 Tat induces viral replications and cellular dysfunctions [3] like inducing oxidative stress, modifying redox, altering pro-inflammation, and various cytokines, chemokines, neurotransmitters and metabolites [4–6]. In addition, HIV-1 Tat regulates apoptosis and activates the intracellular signaling cascade-mediated neurodegeneration [7]. In astrocytes, HIV-1 Tat induces oxidative stress and impairs mitochondrial function, ultimately leading to cell death [8]. Studies show that HIV-1 Tat mRNA expression is increased during HIV brain dementia. However, we do not yet understand the mechanism by which HIV-1 Tat impacts energy resource and oxidative metabolic dysfunction, or how mitochondrial biogenesis and Nrf transcription impact astrocytic energy network-led neurodegeneration.

We are currently facing a global epidemic of psychostimulant abuse, namely cocaine abuse. A significant proportion of cocaine users develop neuronal impairments. Cocaine use can deteriorate CNS cells, including astrocytes, oligodendrocytes, and microglia [9]. Cocaine causes numerous cellular dysfunctions and leads to immune and neuronal disorders, which can subsequently lead to neurodegeneration and cognitive impairments. Cocaine causes inflammation, cytokine, chemokine and cellular metabolism, all of

which affect CNS function [10, 11]. Cocaine use is often associated with HIV infection and can enlarge the major reservoir of viral replication and accelerate the progression of acquired immunodeficiency syndrome (AIDS) [12, 13] which also leads to altered neuronal impairment mediated by oxidative stress induction, energy resources, and fuel transfer [14]. Clinical observations suggest that cocaine use in conjunction with HIV infection may be associated with energy deficits in adenosine triphosphate (ATP) metabolic resources; this results in neuronal impairments in neuroplasticity and the formation of axons and dendrites [15–17].

Brain-energy requirements are very high, and astrocytes are active players in brain-energy resources as they are critically involved in metabolic functions like the supply, production, storage, and utilization of energy to neurons [18–20]. Metabolic enzymes have several cellular functions, including proteolysis and digestion, cellular respiration, energy storage, transcription, and responding to a CNS cell's environmental changes. These energy metabolic life-sustaining pathways are vital for the growth and maintenance of cellular integrity [21]. An increased level of oxidative stress, reactive oxygen species (ROS) production, and redox modification—such as GSH/GSSG ratio—are all associated with metabolic dysfunction [22]. Glutathione (GSH) is a tripeptide that acts as a cell protectant in intracellular antioxidant defense mechanisms. Lower GSH levels have been correlated with an impaired metabolic system [23]. However, oxidative stress and redox modification re-entry seem counterintuitive to oxidative metabolism. Studies show that oxidative stress and redox changes increase ROS-mediated metabolic alterations and are correlated with energy deficits [24]. However, whether oxidative stress response undergoes energy sensor 5' adenosine monophosphate-activated protein kinase (AMPK) activation may depend partly on where the cell resides in the energy deficits.

Several studies have consistently demonstrated that cocaine abuse and HIV infection inhibit energy metabolism and promote the neuronal dysfunction [16, 25, 26]. HIV-infected cocaine users have synergistically potentiated viral replication and disease progression compared to HIV-positive subjects [13, 27, 28]. Despite mounting evidence suggesting that cocaine use aggravates HIV infection, we currently lack mechanistic studies that can determine the mutual role of cocaine and HIV infection on energy metabolism and their role in neurodegeneration. Here, we investigated the mechanisms by which HIV-1 Tat-induced oxidative stress altered energy dysfunction and cocaine-accelerated effects in primary astrocytes. We then validated these mechanistic studies in a HIV- a doxycycline-inducible astrocyte-specific HIV-1 Tat transgenic mouse (iTat) model.

In this study, we investigated the effects of HIV-1 Tat coupled with cocaine-mediated oxidative stress on redox balance via glutathione synthetase (GSS), super oxide dismutase (SOD), catalase (CAT), energy sensor AMPKs and acetyl- CoA (ACC). We sought to examine the effects on the metabolic profiles of hexokinase (HK-I, HK-II), phosphofructokinase (PFK), Lactate Dehydrogenase A (LDHA), the mitochondrial biogenesis of peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α, and the mitochondrial transcription factor (TFAM). We found the levels of nuclear factor erythroid (Nrf) transcription were inhibited, which in turn led to associated neuropathogenesis both *in vitro* and *in vivo* HIV-1 iTat (transgenic mouse model). Cocaine with HIV-1 Tat exacerbated oxidative metabolism and energy

resources shutdown the metabolic switch and affected the energy transfer in astrocyte-mediated neurodegeneration both *in vitro* and *in vivo* of cocaine-exposed with HIV-1 iTat mice.

Methods And Materials

Cell culture and Reagents

We purchased cell-culture reagents from Sciencell (Carlsbad, CA, USA). The GSS, SOD, CAT, AMPK, PGC-1α, TFAM and the goat anti-rabbit IgG, and goat anti-mouse IgG antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). We purchased the AMPKs, ACC, HK-I, HK-II, PFK, LDHA, MCT-1 and MCT-4 antibodies from Cell Signaling (San Jose, CA, USA). The Nrf-1 and Nrf-2 antibodies were purchased from Proteintech (Rosemont, IL). We acquired propidium iodide from BD bioscience (San Jose, CA, USA) and electrophoresis reagents from Bio-Rad (Richmond, CA, USA). We bought nitrocellulose membranes from Amersham Scientific (Piscataway, NJ). All other reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA).

HIV-1 Tat proteins

We obtained the HIV-1 Trans activator (Tat) protein (Cat # 2222) from the National Institutes of Health AIDS Research and Reference Reagent Program. The recombinant Tat proteins had a purity level of > 95%.

Human Primary Astrocytes

We used primary-cell astrocytes isolated from human cerebellum. Astrocytes were maintained in astrocyte basal medium and supplemented with fetal bovine serum to final concentration of 10% and 1% antibiotic/ antimycotic solution, procured from Sciencell (Carlsbad, CA, USA).

Animals and Housing

Adult male HIV-1-inducible transgenic mice (iTat, formerly known as GT-tg bigenic mice [29]) were bred in a colony established at the University of Florida and founded by progenitors that were generously gifted by Dr. Johnny He. All mice (8–10 weeks of age) were maintained at the University of Florida's animal facilities and used in experiments according to protocols approved by the Institutional Animal Care and Use Committee. Procedures for creating HIV-1 iTat mice and confirming the genotype of the inducible and brain-targeted HIV-1 Tat protein are described in detail elsewhere [29, 30].

Drug Treatment

We induced brain-targeted Tat with doxycycline (Dox) treatment, both with and without exposure to cocaine. To express HIV-1 Tat 1–86, iTat mice received Dox via intraperitoneal (i.p.) injections administered daily over 14 days. A single daily dose consisted of 100 mg/kg of Dox dissolved in 0.9% saline, in a volume of 0.3 ml/30 g body weight (iTat-Dox; n = 6). The control group (n = 6) consisted of iTat mice that received saline injections, as described in detail elsewhere [30]. A subset of mice received

subcutaneous (s.c.) administrations of cocaine at a dose of 10 mg/kg/d in 0.9% saline either directly (n = 6) or 2 minutes after the Dox treatment (n = 6).

Isolating brain specimens for gene and immunoblotting analysis

After the 14-day treatment period, mice were anesthetized with isoflurane (4%) until they were euthanized and then subjected to tissue harvest. We collected brains from three euthanized mice from each treatment set, flash froze the tissue in liquid nitrogen, and stored tissues at -80 °C for gene and protein-modification analyses as detailed below.

RNA Extraction and Real-time quantitative PCR (qRT-PCR)

The *in vitro* experiment, we extracted total RNA from primary astrocytes (1×10^6) exposed with HIV-1 Tat (50 ng/ml) and cocaine (0.5 μM) for 24 h based on our previous reports [31]. At the end of the incubation period, cells were split by 0.05% trypsin-EDTA and washed with PBS. The cells were pelleted by centrifugation (1000 × g for 10 min) at room temperature (RT) and suspended in a mitochondrial assay buffer. Cells were incubated in the solution on ice for 2 min and homogenized with a glass homogenizer using 30 up-down strokes. We extracted total RNA from primary astrocytes and HIV-Tat (iTat) mice brain tissues from frontal lobe using the Qiagen kit (Invitrogen Life Technologies, Carlsbad, CA, USA) and following the manufacturer's instructions. We synthesized cDNA using the total RNA (5 μg). We amplified cDNA using specific primers for GSS (Assay ID Hs00609286_m1), AMPK (Hs00272166_m1), catalase (Hs00156308_m1), and housekeeping genes β-actin (Hs99999903_m1; Applied Biosystems, Foster City, CA) served as a control for quantifying real-time PCR. We assessed the relative abundance of each mRNA species using a Q-PCR master mix from Stratagene. This master mix leverages a Mx3000P instrument to detect and plot the increase in fluorescence versus the PCR-cycle number to produce a continuous measure of PCR amplification. We quantitated the relative mRNA species expression and calculated the mean-fold change in expression of the target gene using the comparative CT method (Transcript Accumulation Index, TAI = 2_ΔΔCT). We controlled all data for the quantity of RNA input by performing measurements on an endogenous reference gene, β-actin. In addition, results on RNA from treated samples were normalized to results obtained on RNA from the control, untreated sample.

Mitochondrial Isolation

We used a Mitochondrial Isolation Kit (Abcam, CA) to isolate mitochondria from astrocytes. Briefly, for the *in vitro* experiment, astrocytes (3×10^8) were grown and treated with HIV-1 Tat (50 ng/ml) and cocaine (0.5 μM) for 24 h. At the end of the incubation period, cells were split by 0.05% trypsin-EDTA and washed with PBS. The cells were pelleted by centrifugation (1000 × g for 10 min) at room temperature (RT) and suspended in a mitochondrial assay buffer. Cells were incubated in the solution on ice for 2 min and homogenized with a glass homogenizer using 30 up-down strokes. We confirmed cellular disruption by microscopy. For the *in vivo* experiment, four groups of mice brain frontal lobe tissue (50 mg each) were homogenized: saline, cocaine exposed, HIV-iTat, and cocaine-exposed with HIV-iTat. The extract was centrifuged at 800xg for 10 min. The pellet was a nuclear fraction and the supernatant was centrifuged at

12,000 × g for 15 min at 4 °C. The mitochondrial pellet was then resuspended in lysis buffer for Western blot analysis according the manufacture's protocol.

Western blot analysis

We performed Western blot analysis to determine the redox content of GSS, SOD, catalase, the energy-sensor protein, AMPK, and ACC. In addition, we examined the downstream effect of the glycolytic metabolic profiles of HK-I, HK-II, PMK, LDHA, MCT-1, and MCT-4, mitochondrial PGC-1α, TFAM, and Nrf- protein alterations in primary astrocytes and HIV-iTat mice. Equal amounts of astrocytes and total cellular protein in the brain tissue were resolved on a 4–15% gradient sodium-dodecyl sulfate polyacrylamide-gel electrophoresis (SDS-PAGE). The tissues were then transferred to a nitrocellulose membrane and incubated with their respective primary antibodies. We visualized the immunoreactive bands using a chemiluminescence Western blotting system from Amersham (GE Healthcare Life Sciences, Pittsburgh, PA) and followed the manufacturer's instructions.

Statistical analysis

We performed statistical analysis using GraphPad Prism version 6. We calculated the differences between HIV-1 Tat, cocaine, and HIV-1 Tat with cocaine treated and control using the one-way ANOVA. Values were expressed as mean ± standard error (SE). We used a significance level of $p < 0.05$.

Results

HIV-1 Tat and cocaine shuttle astrocyte energy resources and promote neurodegeneration

HIV infection and HIV-1 proteins are known to induce oxidative stress and redox imbalance, which ultimately causes neurodegeneration [32, 33]. Illicit drugs like cocaine also induce oxidative stress and affect cellular homeostasis. However, we do not yet understand how HIV-1 Tat and cocaine-induced redox modification leads to energy deficits, how energy metabolism affects astrocytic networks, or how these changes lead to neurodegeneration. Therefore, we determined whether HIV-1 Tat induce redox modification, energy metabolism, mitochondrial biogenesis leading neurodegeneration. Ultimately, we sought to determine how these effects are accelerated by cocaine.

Our findings demonstrate that HIV-1 Tat and cocaine 1) significantly affects the GSS and CAT and increased the level of SOD; and 2) activated AMPK expression leading metabolic enzymes and mitochondrial biogenesis in human primary-astrocytes culture. In addition, the validation analysis by HIV-iTat mice brain total and mitochondrial fractions observed results are mimicked in similar and confirmed the *in vitro* pattern.

HIV-1 Tat and cocaine-effect redox modification, energy metabolic profiles gene and protein expression in human primary astrocytes

Figure 1 shows the effects of HIV-1 Tat, cocaine, and HIV-1 Tat with cocaine exposures impact on redox gene GSS (A), CAT (B), and energy sensor AMPK expression (C). These results indicate that HIV-1 Tat and cocaine exposure inhibit the levels of GSS and CAT whereas AMPK expression significantly increased. Moreover, the combination of HIV-1 Tat with cocaine accelerated these effects compared to control. The present finding suggests that HIV-1 Tat with cocaine 1) increases oxidative stress by decreasing GSS and CAT, and 2) increases AMPK compared to either HIV-1 Tat or cocaine exposure alone *in vitro* (Fig. 1. A-C) and *in vivo* (Fig. 1D-F).

Furthermore, we examined whether HIV-1 Tat-induced oxidative stress and redox protein regulation is accelerated by cocaine. Figure 2 shows the effects of three treatment groups—HIV-1 Tat, cocaine alone, and HIV-1 Tat with cocaine—on redox protein GSS (A), SOD (B), CAT (C), energy sensor AMPK (D), and ACC (E). The densitometry analysis is shown in Fig. 2 for GSS (F), SOD (G), CAT (H), P-AMPK (I), and P-ACC (J). These results confirm that HIV-1 Tat-induced redox changes were accelerated by cocaine. Taken together, these findings suggest that HIV infection altered redox imbalance and activate energy sensor AMPK. Moreover, the addition of cocaine affects astrocytic gene- and protein-expression signaling cascades.

HIV-1 Tat and cocaine impact oxidative metabolism and glycolytic profiles in astrocytes

Reduced the level of GSH is known to affect cellular functions such as intracellular signaling networks, which are mediated by several cellular consequences of neurotransmitters and ion channels; furthermore, metabolic profiles control the energy resources of glucose storage and energy transfer level [34]. Therefore, we wanted to test whether exposure to HIV-1 Tat affects astrocytic energy resources and leads to cellular oxidative metabolism in glycolytic enzyme-mediated neurodegeneration. In Fig. 3, HIV-1 Tat with cocaine-treated primary astrocytes (for 24 h) showed significant reductions in HK-I. By contrast, HK-II, PYK1/2, LDHA, and MCT-1 and MCT-4 showed significantly increased protein expression levels at 24 h compared with either HIV-1 Tat or cocaine alone. Figure 3 shows the effects of HIV-1 Tat with cocaine on HK-I (A), HK-II (B), PKM1/2 (C), LDHA (G), MCT-1 (H) and MCT-4 (I) protein expression. We can directly compare these results with the control and the densitometry analysis in Fig. 3; see HK-I (D), HK-II (E), PKM1/2 (F), LDH (J), MCT-1 (K), and MCT-4 (L) respectively. These protein activations confirmed changes in the metabolic profile of glycolytic enzymes and underscored that cocaine accelerated the impact on astrocytic energy networks.

HIV-1 Tat and cocaine accelerates energy deficits and leads to mitochondrial biogenesis and Nrf transcriptions

HIV infection affects astrocyte energy storage, utilization, and transfer of energy resources, and ultimately leads to CNS dysfunctions [25, 35–37]. Astrocytes are the major source of energy storage sites of glycogen granules in the CNS. HIV infection are known to target the brain-energy environment and subsequently affect the cellular functions in the CNS. Therefore, we examined how HIV-1 Tat with cocaine affect energy resources and oxidative metabolism and the downstream impact on mitochondrial biogenesis-mediated astrocyte signaling networks. Figure 4 shows that HIV-1 Tat with cocaine-treated astrocytes significantly upregulated PGC-1α and the mitochondrial transcriptional factor TFAM as compared with either HIV-1 Tat or cocaine alone. This result was consistent in primary astrocytes cytoplasm (Fig-4 A and B), mitochondrial fraction (Fig-4, E and F), densitometry analysis of PGC-1α and TFAM in cytoplasm (Fig-4, C and D), and in mitochondria (Fig-4, G and H). In addition, HIV-1 Tat/cocaine increased the level of oxidative phosphorylation (OXPHOS; Fig – 4, I and J), which impacts oxidative metabolism and is led by PGC-1α and TFAM, which target Nrf transcription. However, Nrf transcriptional factors bind to the antioxidant-gene elements and control oxidative stress, which are mediated by Nrf transcription. Therefore, we investigated Nrf transcriptional factors 1 and 2 using HIV-1 Tat with cocaine. Interestingly, our results showed that Nrf-1 and Nrf-2 are differentially regulated; that is, Nrf-1 is significantly down regulated, and Nrf-2 is significantly upregulated in the cytoplasm and subsequently decreased in mitochondria fractions (Fig-4 K-N).

HIV-1 iTat and cocaine impact oxidative metabolism and glycolytic profiles

Our validation analysis (shown in Fig. 5) of the *in vivo* HIV-1 iTat mice model suggests that oxidative stress-induced redox imbalance inhibits GSS (A) and catalase (E), which subsequently increases the level of SOD1 (C) and the astrocytic marker of GFAP (G); it also activates the energy sensor AMPKs proteins (I). Figure 6, panels B, D, F, H, and J show the densitometry analyses of GSS, SOD1, catalase, GFAP, and AMPK expression, respectively. The energy sensor AMPK is known to alter glycolytic metabolic enzymes, which are the validated analysis from cocaine-exposed with HIV-iTat mouse brain. Figure 6 demonstrates that HK-II (A), LDH (C), PYK (E) and PMK1/2 (G) are increased by HIV-iTat. Moreover, these effects are accelerated by cocaine. Figure 6, panels B, D, F, and H show the densitometry analysis of HK-II, LDH, PYK, and PMK1/2, respectively.

HIV-1 iTat and cocaine lead oxidative phosphorylation, mitochondrial biogenesis and NRF transcription

Energy resource, transfer, and utilization are maintained by oxidative metabolism. Glycolytic enzymes are the major players in oxidative metabolism; changes in glycolytic enzymes are known to activate OXPHOS-mediated mitochondrial biogenesis. The validation analysis of *in vivo* cocaine-exposed HIV-iTat mice synergistically accelerated OXPHOS compared to control (Fig. 7A and B) and led to mitochondrial biogenesis. Figure 7 shows that unlike either HIV-iTat or cocaine alone, in the cocaine-exposed with HIV-iTat condition, PGC-1α and TFAM were upregulated in the cytoplasm and mitochondrial fraction

compared to control (Fig. 7. C- F). These data confirm that mitochondrial biogenesis of PGC-1α and TFAM are activated by OXPHOS, which may impact Nrf transcription. However, Nrf transcription is known to control the antioxidant responsive elements (ARE) of mitochondrial genes [38]. Figure 7 (G – J) shows that the level of inhibition in Nrf- 1 (Figure G – H) and Nrf- 2 (I - J) may promote neurodegeneration. This effect might be accelerated by cocaine comorbidity. However, these two isoforms of Nrf-1 and Nrf-2 are similar in HIV-Tat with cocaine exposure in *in vitro.* In fact, our validation analysis in *in vivo* HIV-1 iTat/cocaine confirms these effects.

Discussion

HIV infection and illicit drugs are known to induce oxidative stress and ROS production, which can ultimately impact cellular functions like oxidative metabolism and cause energy deficits [25, 39–41]. GSH plays an important cell-protectant role by exhibiting an antioxidant defense mechanism. It is closely linked to the energy source of ATP production. Low levels of GSH have been associated with impaired immune and neuronal dysfunction; its metabolites also interface with energetics and neurotransmitter syntheses through several prominent metabolic pathways [42, 43]. HIV infection affects redox expression in GSS, SOD and catalase, as evidenced in HIV-infected patients and cocaine users [44–46]. However, inhibition of redox imbalance GSH/GSSG [47] activates energy sensor AMPKs and subsequently impacts the energy-profile machinery of metabolic enzymes such as HK-1, HK-II PKM, LDH, MCT-1, and MCT-4, all of which play vital roles in neuronal dysfunction and in the disease progression of HIV-associated dementia (HAD) patients [48]. In CNS, astrocytes are the major reservoirs of energy storage and maintain cellular energy metabolism. Astrocytes are modulated by HIV infection as well as drug use, both of which lead to neurodegeneration [12, 33, 49]. HIV-1 Tat impacts several cellular functions, but we do not yet understand the effects of HIV-1 Tat and cocaine-induced redox imbalance on energy deficits, oxidative metabolism, and ultimately, neurodegeneration. Our observations provide new insights into the functional role of redox imbalance, which adequately alters the energy profiles of glycolytic enzymes and mitochondrial biogenesis, which leads to Nrf transcription and is a potential sign of neurodegeneration by HIV-1 Tat with cocaine.

We have demonstrated for the first time that HIV-1 Tat, cocaine, and HIV-1 Tat with cocaine led to redox inhibition of GSS and catalase and subsequently increased SOD and activation of the energy sensor AMPK and mRNA expression (Fig-1 and 2), as compared with the control. It's known that redox imbalance disturbs cellular homeostasis [34]. Our study suggests that cocaine with HIV–1 Tat may play an enhanced role in oxidative metabolism compared to the control. Our results are consistent with earlier reports of HIV-gp120-induced microglia and HIV-Tat-induced astrocytes, where activation of the redox and oxidative pathways were observed [25, 26]. AMPK is a key regulator of metabolism and survival during energy stress. Dysregulation of AMPK is strongly associated with oxidative injury, which impacts redox modification. Defective oxidative metabolism and a reduced level of ATP are both known to activate AMPK [50]. AMPK plays a critical role in controlling both cellular and whole-body metabolic responses. However, we still do not know how HIV-1 Tat and cocaine regulate AMPK, which can impact intracellular redox (GSS, CAT and SOD) status. These studies confirm that redox imbalance and energy dysfunction

affect glycolytic enzymes and mitochondrial biogenesis. These factors, in turn, activate OXPHOS and impair Nrf transcriptions, which ultimately leads to astrocytic signaling mediated neurodegeneration.

Our results also show that in cocaine, HIV-1 Tat, and cocaine with HIV-1 Tat, induction of redox inhibition and activation of AMPK is associated with altered glycolytic enzymes and increased mitochondrial biogenesis. Importantly, HIV-1 Tat, cocaine, and cocaine with HIV-Tat all led to oxidative metabolism, which in turn impaired mitochondrial biogenesis and subsequently reduced the level of energy resource and transfer. However, HIV-positive cocaine users have higher energy demands which increase ATP utilization and subsequently affect glycolytic enzymes. This study suggests that HIV-1 Tat-induced redox inhibition and energy demands are accelerated by cocaine use when compared with either cocaine use or HIV infection. These results confirm previous reports of HIV-1 Tat-induced metabolic profiles [26].

Previous studies have also demonstrated that HIV infection and HIV proteins affect oxidative metabolism and glycolytic enzyme regulation, which may impact mitochondrial biogenesis [51]. The first step in the breakdown of glucose is to extract energy for cellular metabolism. Glucose phosphorylation is catalyzed by the enzyme HK, which is the predominant isoform of HK I and HK II. Importantly, HK I and HK II contain a hydrophobic terminal mitochondrial binding motif. The energy metabolism in glycolysis is mainly thought of as a cytosolic process and HK I is predominantly associated with mitochondria in ATP production. In contrast, HK II is located in either the cytosol or at the mitochondrial outer membrane [52]. Interestingly, our results demonstrated that HIV–1 Tat and cocaine exposure significantly down-regulated HK I and upregulated HK II expression; moreover, these effects were accelerated by cocaine combined with HIV-1 Tat. These results confirm that increased HK II expression and its binding to mitochondria facilitate and increase the level of aerobic glycolysis and lactate production. However, the increased level of HK- II targets mitochondria-mediated cell death, which is known to activate LDH. Furthermore, monocarboxylic acid transport (MCT) is one of the metabolic targets wherein the flux of small ketone bodies such as lactic acid and pyruvic acid occurs to support metabolic demands. The predominant role of MCTs 1–4 is the transport of L-lactate, pyruvate, and ketone bodies in and out of cells; L-lactate is quantitatively the most important substrate [53]. MCT1 is responsible for the efflux of lactic acid when oxygen supply is compromised, and glycolysis is stimulated. However, glycolytic profiles in astrocytes treated HIV-Tat and cocaine significantly upregulated MCT1 and MCT4, which may increase lactic acid and initiate neurodegeneration. Recent studies conclude that in the brain, MCT1 and MCT4 export astrocyte-produced lactic acid, which is then taken up by MCT1 or MCT2 into the neurons for oxidation as an important respiratory fuel [54].

The energy sensor AMPK is key regulator that activates PGC-1α, a member of the peroxisome proliferator-activated receptor-gamma (PGC) family of transcriptional coactivators and is the master regulator of mitochondrial biogenesis [55]. AMPK activates different transcriptional factors, which promote the expression of TFAM, including Nrf-1 and Nrf-2. The oxidative stress response is increased by antioxidant defenses through the activation of Nrf, an important transcription factor [56, 57]. Nrf is the main player in the controlled antioxidant-response element (ARE) found in the promoter regions of many genes that encode antioxidants and detoxification enzymes (e.g., SOD1, CAT, and GPx1) [38]. In addition, Nrf-1 and

Nrf-2 are important contributors to the sequence of events that increase the transcription of key mitochondrial enzymes. Nrf-1 and – 2 can interact with TFAM, which drives the transcription and replication of mtDNA [58]. TFAM is a downstream target gene of PGC-1α and controls the transcription of mitochondrial DNA-encoded genes as well as DNA replication during biogenesis [59].

In the present study, exposure to HIV-Tat with cocaine led to the activation of PGC-α1 (unlike the control group). The analysis of the level of TFAM protein in total and mitochondrial fraction was increased by exposure of HIV-1 Tat or cocaine alone. Compared to control, these effects were accelerated by HIV-Tat with cocaine co-morbidity in total cell lysates as well as a mitochondrial fraction. Other studies have confirmed that mitochondrial damage induced by increasing mitochondrial biogenesis depends on upregulated PGC-1α expression [60, 61]. Also, increased oxidative damage and the level of PGC-1α and TFAM in astrocytes could enhance the susceptibility of mtDNA, which might cause neuronal dysfunction and result in neurodegeneration. Therefore, it is possible that the impact of energy consumption during HIV infection inhibits redox expression and dysregulates AMPK-mediated metabolic function and mitochondrial impairments, thus leading to Nrf transcription. Several studies show that interrupting glycolysis can contain oxidative metabolism but has an adverse effect on CNS function that is out of proportion with any change in total-energy status [62, 63]. In addition, clinically significant energy imbalance may also occur as a result of an important energy-generating enzyme system in mitochondria. In support to our study a recent report demonstrated that mitochondrial biogenesis is differentially regulated in neurons and astroglia in HIV associated neurocognitive disorders (HAND) brains and that targeting astroglial bioenergetics processes [64]. Taken together, these data indicate that PGC-1α plays a crucial role in linking stimuli such as HIV infection and cocaine abuse to an internal metabolic response like mitochondrial biogenesis via, among others, the NRF transcription factors.

Overall, the data suggest a connection between the redox gene and protein enhancing the activation of AMPKs and altered intracellular signaling mechanisms in cocaine and HIV- Tat treated cells. The upregulation of redox-gene inhibition and altered oxidative metabolism by cocaine and HIV-Tat protein may lead to increased energy fuel utilization and ultimately affect the storage of energy resources and transfer metabolic fuel which can lead to cell death. The present study supports the idea that oxidative stress and redox-dependent gene expression are associated with energy dysfunction, particularly with the reduction of glutathione (GSH) and the activation of energy sensor AMPK, glycolytic enzymes, and mitochondrial biogenesis. The contained medium exposed studies show that in SK-N-MC neuroblastoma cells there was a loss in the number of spines, and a decrease in dendrite diameter, dendrite area, and spine area compared to control. These results suggest that either cocaine or HIV-infected astrocytes play a vital role in energy storage, metabolic dysfunction, and neurodegeneration.

Summary And Conclusion

To the best of our knowledge, this study reveals that HIV Tat-inducing redox imbalance activates the energy sensor AMPK, which increases the level of energy deficit, mitochondrial biogenesis, and Nrf transcription. The metabolic switch, in turn, affects the energy-transfer process to neurons in HIV-1 Tat

with cocaine. Based on these results, redox imbalance potentiates HIV viral replication and disease progression by impairing the astrocyte metabolic function, which may lead to neurodegeneration. The present study supports some of earlier reports in the dysfunction of energy metabolism and increased neurodegeneration in cocaine with HIV infection and HIV-derived protein [25, 26].

Abbreviations

iTat - HIV-1 inducible Tat (transgenic mice)

HIV-1 Tat- Trans activator protein

ROS- Reactive oxygen species

GFAP- glial acidic fibril protein

GSS - glutathione synthetase

CAT- Catalase

SOD - super oxide dismutase

HAND- HIV-associated neurocognitive disorders

AMPK - 5' AMP-activated protein kinase

HK- Hexo kinase

ACC- Acetyl coenzyme A

PFK- *Phosphofructokinase*

LDHA- Lactate dehydrogenase

MCT- Monocarboxylate transporters

PGC1α -Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

TFAM- transcription factor A, mitochondrial

CaMK -Ca2+/calmodulin-dependent protein kinase

CREB- cAMP response element-binding protein

ACC- Acetyl-CoA carboxylase

OXPHOS- Oxidative phosphorylation

NRF- Nuclear respiratory f*actor*

ARE- Antioxidant response element

Declarations

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics Statement

The studies involving mouse were reviewed and approved by the Ethics Committee of University of Florida.

Author's Contributions

TS participated in the design of the study and wrote the paper; KS carried out the gene and protein experiments. TJC and JPM participated and performed the mouse experiments.

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Consent for publication

Not Applicable

Conflict of interest

The authors declare that they have no conflicts of interest.

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Figures

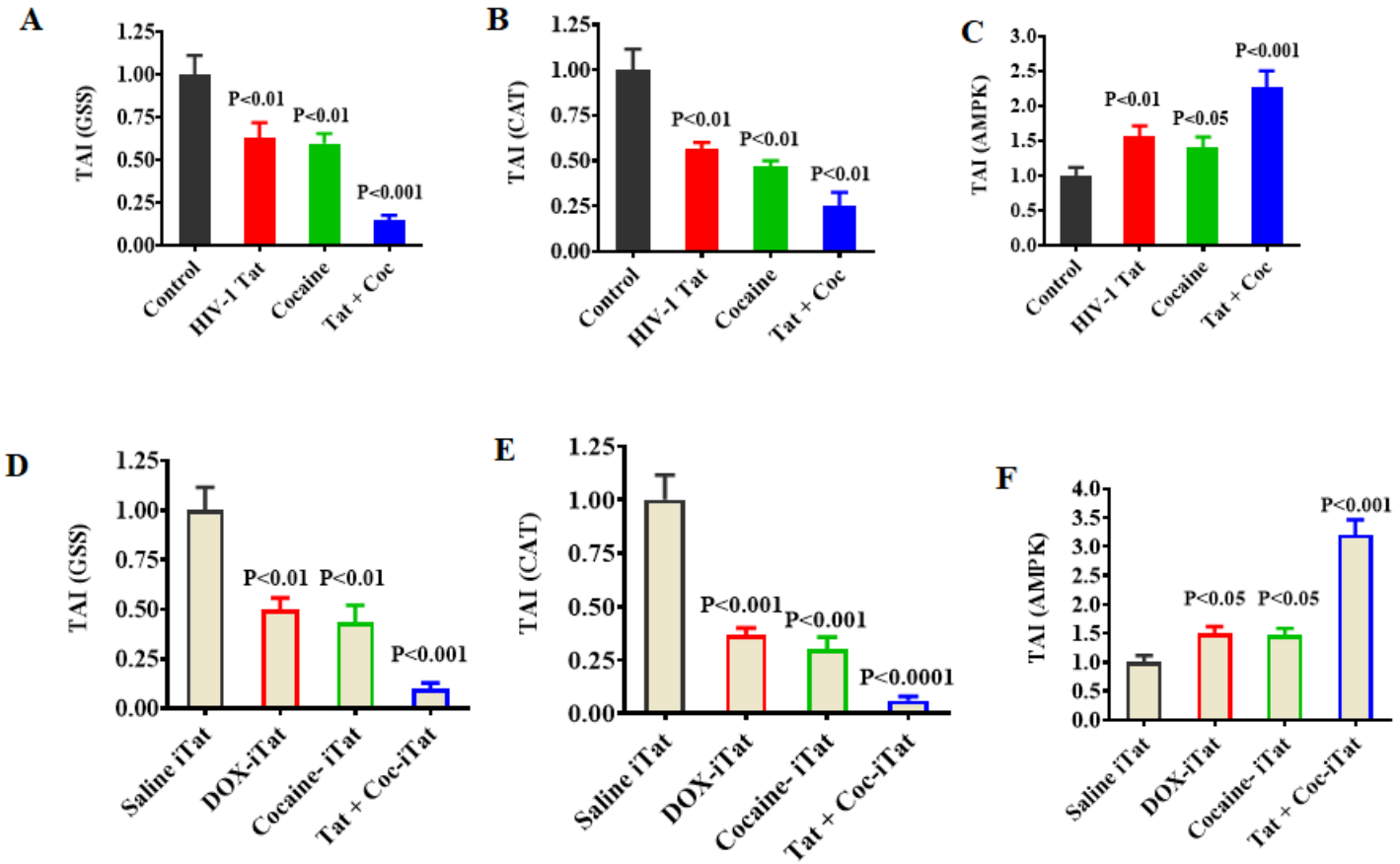

Figure 1

Effect of HIV-1 Tat and cocaine on redox and AMPK-gene expression in in vitro and in vivo experiments. Primary human astrocytes (A, B and C; 1 x 106 cells/ ml) were treated with HIV-1 Tat (50 ng), cocaine (0.5 μM) or HIV-1 Tat with cocaine for 24 h. The controls were maintained in a drug-free medium. HIV-1 transgenic (iTat) mice (D, E and F) were treated for 14 days with a daily i.p. injection of either saline or doxycycline (Dox) 100 mg/kg/d, with or without s.c. injections of 10 mg/kg/d cocaine, administered consecutively at 24 h intervals for 14 days. At the end of treatment, we harvested astrocytes and brain tissues, extracted RNA, and reverse transcribed. Next, we conducted quantitative real-time PCR for in vitro

GSS (A), CAT (B), AMPK (C), in vivo GSS (D), CAT (E) and AMPK (F). Data are expressed as mean ± SE of TAI values of five independent experiments.

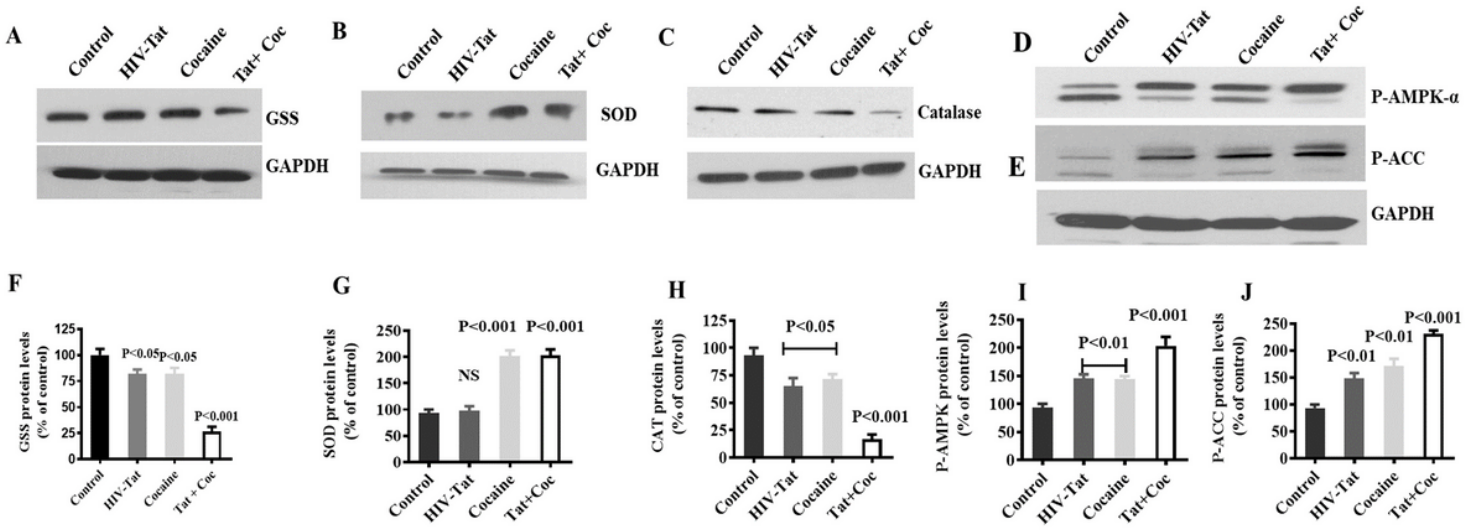

Figure 2

Effect of HIV-1 Tat and cocaine on redox proteins modification. Primary human astrocytes (1 x 106 cells/ml) were treated for 24 h with either HIV-1 Tat (50 ng), cocaine (0.5 μM), or a combination of HIV-1 Tat with cocaine. Controls were maintained in drug-free medium. At the end of the incubation, an equal amount of protein lysate was resolved by 4-15% SDS-PAGE. We analyzed protein expression using Western blot showing GSS (A), SOD (B), CAT (C), AMPK (D) and ACC (E). Panels F, G, H, I and J represent the percent of densitometric values of protein levels (% control). Data are expressed as mean ± SE of five independent experiments.

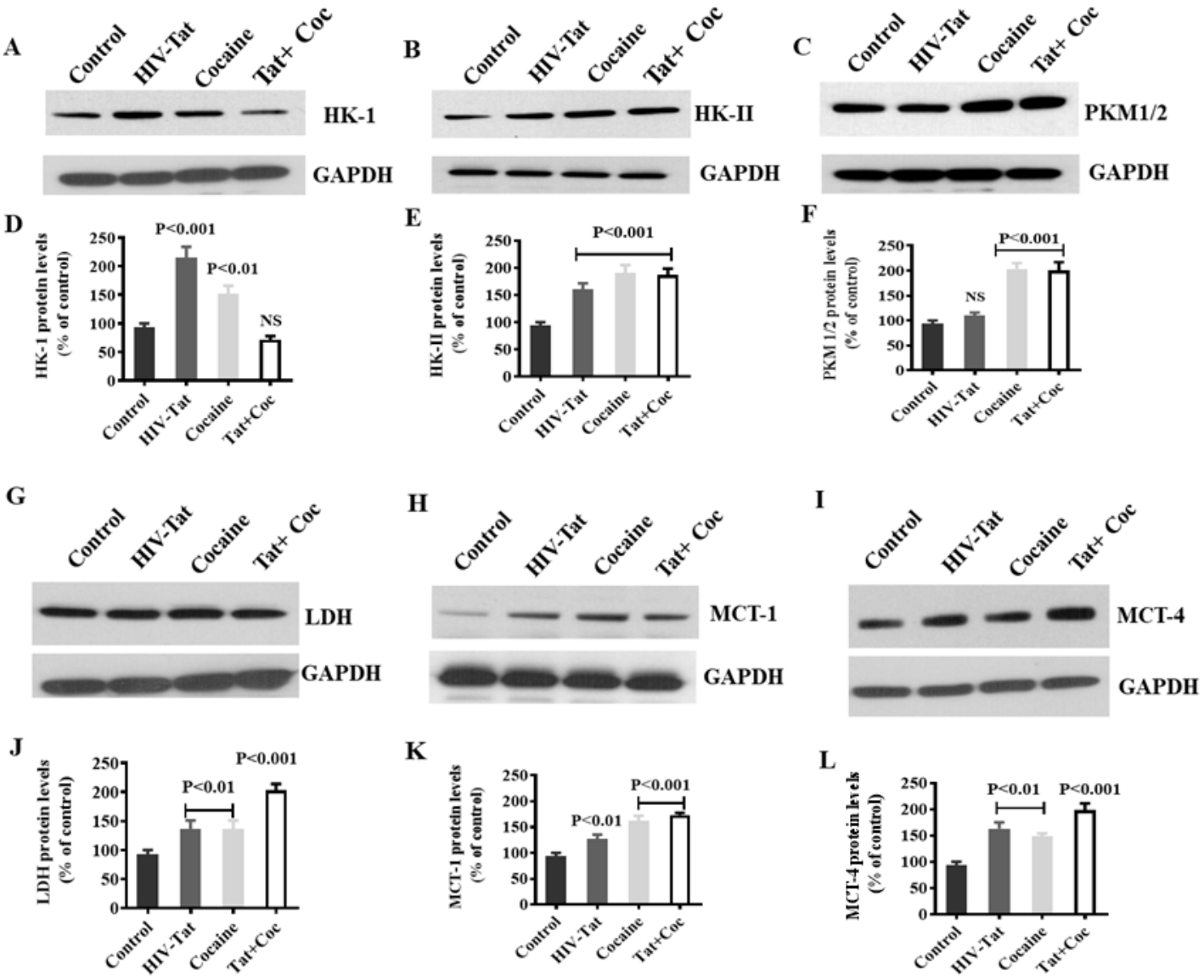

Figure 3

Effect of HIV-1 Tat and cocaine on the modification of energy metabolic glycolytic profiles. Primary human astrocytes (1 x 106 cells/ ml) were treated for 24 h with HIV-1 Tat (50 ng), cocaine (0.5 μM), or a combination of HIV-1 Tat with cocaine. Controls were maintained in a drug-free medium. At the end of the incubation, an equal amount of protein lysate was resolved and protein expression was analyzed using Western blot showing HK-I (A), HK-II (B), PKM1/2 (C), LDH (G), MCT-1 (H), and MCT-4 (I). Panels D, E, F J, K, and L represent the percent densitometric values protein levels (% control). Data are expressed as mean ± SE of five independent experiments.

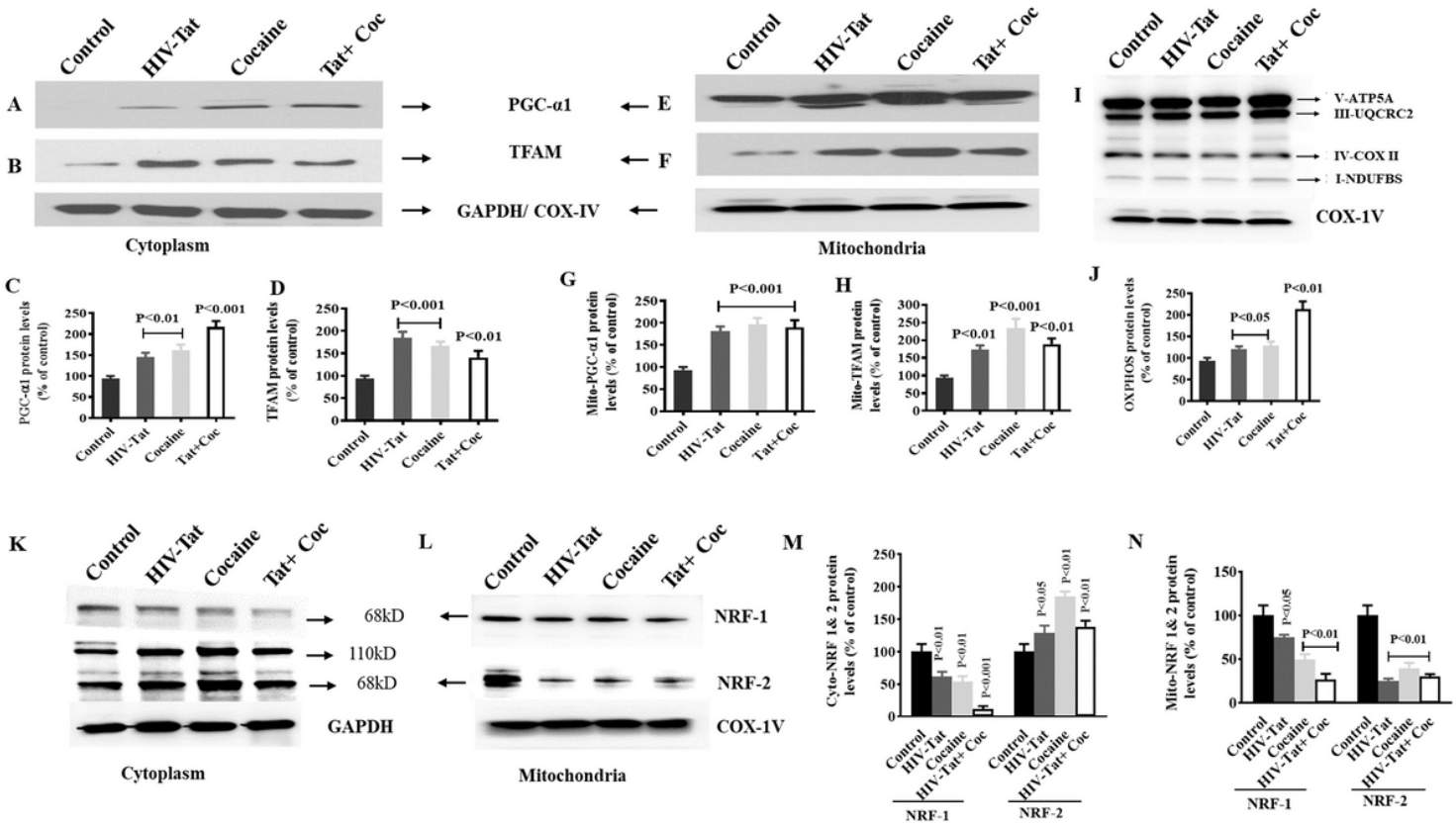

Figure 4

Impact of HIV-1 Tat and cocaine on mitochondrial biogenesis. Primary human astrocytes (1 x 106 cells/ml) were treated with HIV-1 Tat (50 ng), cocaine (0.5 μM), and a combination of HIV-1 Tat with cocaine for 24 h. Controls were maintained in a drug-free medium. At the end of incubation, an equal amount of protein lysate was resolved and protein expression was analyzed using Western blot showing cytoplasmic PGC-1α (A), TFAM (B), mitochondrial PGC-1α (E), AFTM (F), OXPHOS (I), cytoplasmic NRF1 and 2 (K), and mitochondrial NRF1 and 2 (L). Panels C, D, G, H, J, M and N represent the % densitometric values protein levels (% control). Data are expressed as mean ± SE of three independent experiments.

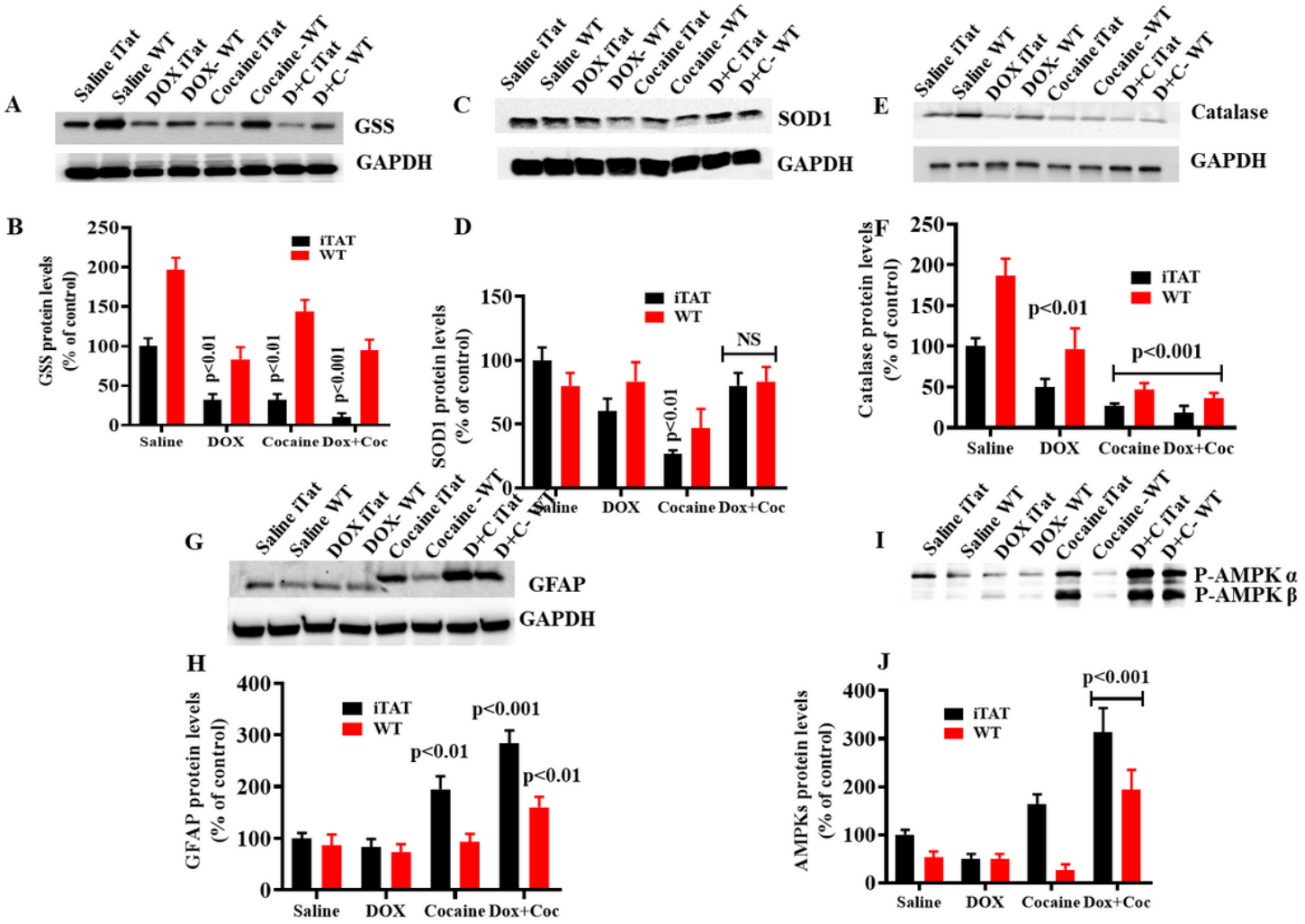

Figure 5

Impact of HIV-1 Tat and cocaine on redox expression and energy-sensor protein in vivo. HIV-1 inducible Tat transgenic (iTat) or saline-administered (control) mice received daily intraperitoneal injections of saline or doxycycline (Dox; 100 mg/kg/d) over 14 days, either with or without s.c. cocaine (10 mg/kg/d). Following treatment, an equal amount of protein lysate from harvested brains were resolved and we analyzed protein expression using Western blot showing GSS (A), SOD (C), CAT (E), GFAP (G), and AMPK (I). Figure B, D, F H, and J represent the percent densitometric values protein levels (% control). Data are expressed as mean ± SE of three independent experiments.

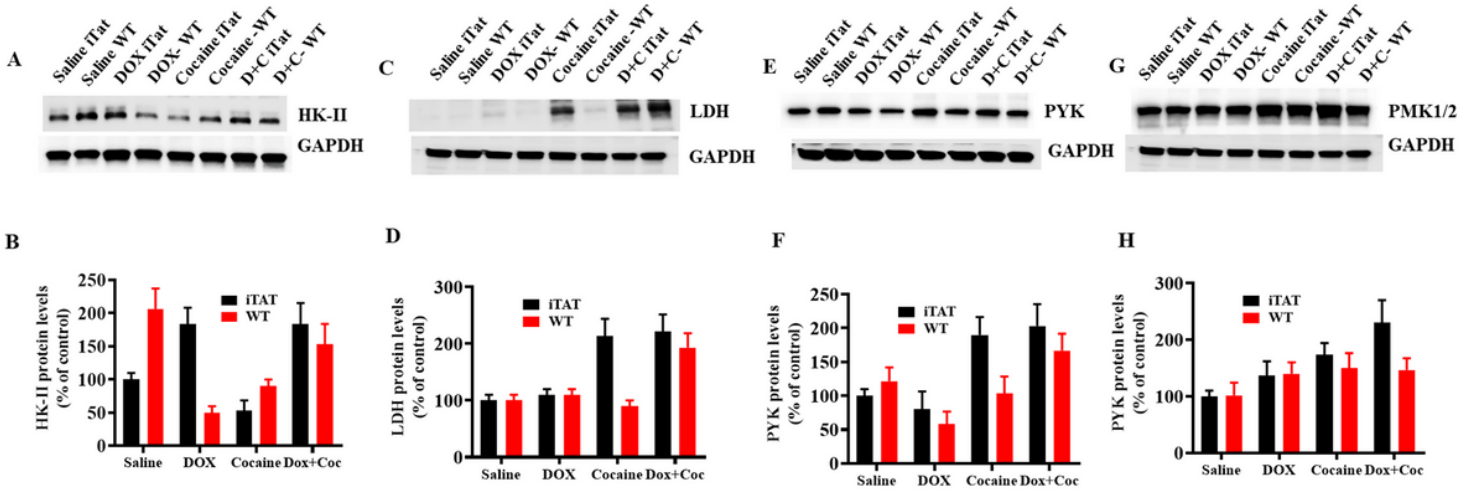

Figure 6

Investigating the effects of cocaine and HIV on the energy metabolic profiles of the glycolytic enzyme in HIV-transgenic (iTat) mice. HIV-1 inducible Tat transgenic (iTat) mice received daily i.p. injections of saline or doxycycline (Dox; 100 mg/kg/d) over 14 days, either with or without subcutaneous cocaine (10 mg/kg/d). Following treatment, we resolved an equal amount of protein lysate from harvested brains by 4-15% SDS-PAGE. We analyzed protein expression using Western blot showing HK-II (A), LDH (C), PKM (E), and PYK1/2 (G). Figure B, D, F and H represented percent densitometric values protein levels (% control). Data are expressed as mean ± SE of three independent experiments.

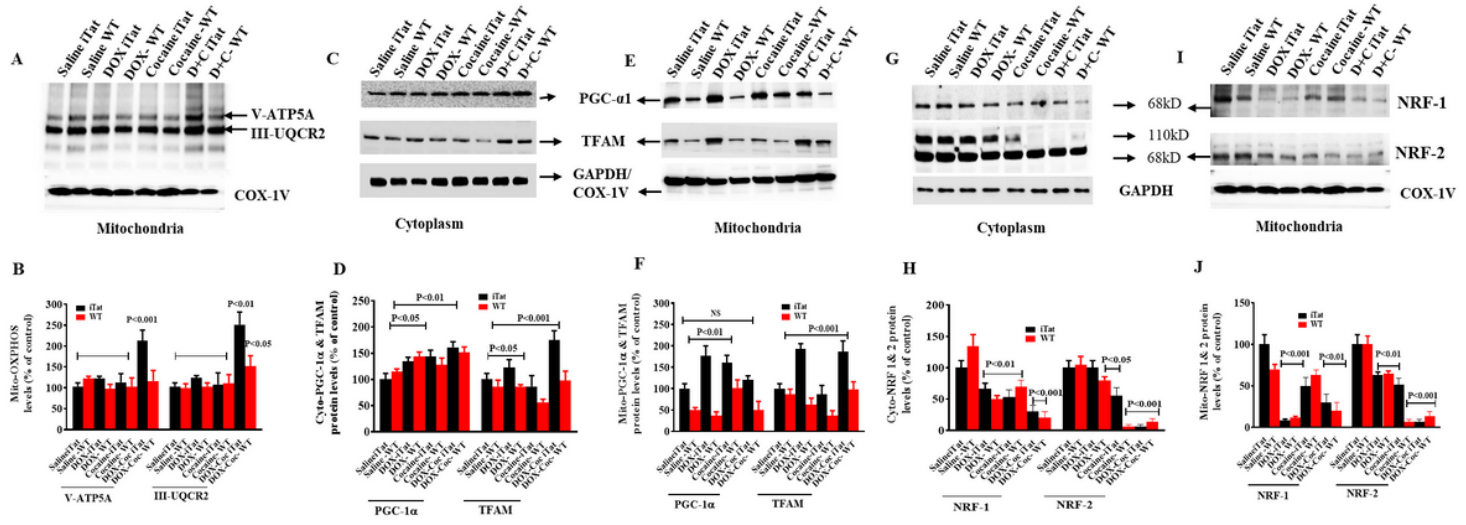

Figure 7

Impact of HIV-1 Tat and cocaine on mitochondrial biogenesis and OXPHOS-impaired Nrf transcription. HIV-1 Transgenic (iTat) mice received daily i.p. injections of saline or doxycycline (Dox; 100 mg/kg/d) over 14 days, either with or without s.c. cocaine (10 mg/kg/d). The end of the treatment, an equal amount of protein lysate was resolved by 4-15% SDS-PAGE. We analyzed protein expression using Western blot

showing cytoplasmic PGC-1α (C), TFAM (C) and mitochondrial PGC-1α, TFAM (E), OXPHOS (A), cytoplasmic NRF1 and 2 (G) and mitochondrial NRF1 and 2 (I). Figure B, D, F, H, and J represented % densitometric values protein levels (% control) respectively. Data are expressed as mean ± SE of three independent experiments.