J. Neurovirol.
DOI 10.1007/s13365-015-0403-6

Decreased glial and synaptic glutamate uptake in the striatum of HIV-1 gp120 transgenic mice

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Received: 8 May 2015 / Revised: 27 October 2015 / Accepted: 30 October 2015

Abstract The mechanisms leading to the neurocognitive deficits in humans with immunodeficiency virus type 1 (HIV-1) are not well resolved. A number of cell culture models have demonstrated that the HIV-envelope glycoprotein 120 (gp120) decreases the reuptake of glutamate, which is necessary for learning, memory, and synaptic plasticity. However, the impact of brain HIV-1 gp120 on glutamate uptake systems in vivo remains unknown. Notably, alterations in brain glutamate uptake systems are implicated in a number of neurodegenerative and neurocognitive disorders. We characterized the kinetic properties of system X_{AG} (sodium-dependent) and systems x_{c-} (sodium-independent) [3H]-L-glutamate uptake in the striatum and hippocampus of HIV-1 gp120 transgenic mice, an established model of HIV neuropathology. We determined the kinetic constant V_{max} (maximal velocity) and K_m (affinity) of both systems X_{AG} and x_{c-} using subcellular preparations derived from neurons and glial cells. We show significant (30–35 %) reductions in the V_{max} of systems X_{AG} and x_{c-} in both neuronal and glial preparations derived from the striatum, but not from the hippocampus of gp120 mice relative to wild-type (WT) controls. Moreover, immunoblot analysis showed that the protein expression of glutamate transporter subtype-1 (GLT-1), the predominant brain glutamate transporter, was significantly reduced in the striatum but not in the hippocampus of gp120 mice. These extensive and region-specific deficits of glutamate uptake likely contribute to the development and/or severity of HIV-associated neurocognitive disorders. Understanding the role of striatal glutamate uptake systems in HIV-1 gp120 may advance the development of new therapeutic strategies to prevent neuronal damage and improve cognitive function in HIV patients.

Keywords Glutamate transporters · gp120 · HIV-1 · Striatum · Cognitive deficits

Introduction

The mechanisms leading to the neurocognitive deficits in humans with immunodeficiency virus type 1 (HIV-1) are not well understood. Glutamate is the most abundant neurotransmitter in the brain and is necessary for learning, memory, and cognitive processing (Danbolt 2001). Notably, glutamate's accumulation in the extracellular space must be tightly regulated by glutamate transporters, which are necessary for maintaining fast neuronal glutamate transmission and synaptic plasticity and preventing neurotoxicity (Huang and Bergles 2004; Matsugami et al. 2006). The HIV-1 genome codes for a number of envelope proteins such as glycoprotein 120 (gp120) that cause major disturbances in neuronal function, often leading to extensive synaptodendritic injury (Kaul et al. 2001; Ellis et al. 2007). In particular, a number of studies using cell culture models have shown that gp120 significantly reduces the expression and function of glutamate transporters, resulting in excessive accumulation of extracellular glutamate, overstimulation of glutamate receptors, and neurotoxicity (Dreyer and Lipton 1995; Vesce et al. 1997; Patton et al. 2000; Belmadani et al. 2001; Wang et al. 2003). Although considerable effort has been expended to understand the mechanisms responsible for glutamate transporter dysfunction in vitro, there are no

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Published online: 13 November 2015

studies available that have determined the effects of gp120 on glutamate uptake systems in vivo.

Two types of transporter systems are necessary for maintaining brain extracellular glutamate homeostasis. These include a family of sodium-dependent and sodium-independent glutamate transporters, referred to as systems X_{AG} and x_{c-}, respectively (Danbolt 2001; Kanai and Hediger 2003). System X_{AG} accounts for the majority (~90 %) of total glutamate uptake in the central nervous system (CNS). The primary excitatory amino acid transporter (EAAT) for system X_{AG} is glutamate transporter subtype 1 (GLT-1/EAAT2), which is ubiquitously expressed in the brain and predominantly in astrocytes (Danbolt 2001). System x_{c-}, however, acts as an obligate exchanger of intracellular glutamate for extracellular cystine, where cystine is transported into cells and reduced to cysteine for glutathione synthesis (Cho and Bannai 1990; Bridges et al. 2012). Notably, disruption of system x_{c-} could lead to elevated intracellular concentrations of glutamate, which may result in cell death due to oxidative stress (Murphy et al. 1989; Patel et al. 2004). Deficits in either of these glutamate uptake systems are implicated in a number of neurodegenerative and neurocognitive disorders ranging from Alzheimer's disease to drug addiction (Choi 1988; Gonzalez and Robinson 2004; Kalivas 2009; Bridges et al. 2012).

In the present study, we characterized the kinetic properties of system X_{AG} and x_{c-} in the striatum and hippocampus of HIV-gp120 transgenic mice, a well-established rodent model of HIV-associated neurocognitive deficits (Toggas et al. 1994). Gp120 mice are shown to manifest dendritic pruning and neuronal loss in various brain regions, particularly the striatum, which appears particularly vulnerable to gp120 (Toggas et al. 1994; Ellis et al. 2007). We revealed a significant deficit in the V_{max} (maximal velocity) of both systems X_{AG} and x_{c-} in the striatum of gp120 mice relative to wild-type (WT) controls and without altering the K_m (affinity) for glutamate. There were no significant alterations observed in the hippocampus. In separate cohorts, we also demonstrated that the V_{max} of system X_{AG} and x_{c-} were reduced in both glial and neuronal preparations from the striatum of gp120 mice, extensive deficits that likely contribute to the onset and/or severity of HIV-associated neurocognitive disorders.

Materials and methods

Subjects

A breeding colony of homozygous transgenic mice producing HIV-1 gp120 in their brains under the regulatory control of a modified murine glial fibrillary acidic protein (GFAP) gene (described in Toggas et al. 1994) were maintained in our animal facility along with B6SJLF mice (cross between C57BL/6J female × SJL/J male), which were used as wild-type (gp120 background) controls. All breeder stocks were purchased from the Jackson Laboratories (Bar Harbor, ME). Following weaning, animals were housed in clear plastic ventilated cages, maintained in a temperature- and humidity-controlled room on a 12-h light/dark cycle with food and water provided ad libitum. Only adult male gp120 and WT mice were used for the experiments. For brain preparations, mice were removed from their home cages and killed by decapitation using sharp-ended scissors. The brains were rapidly extracted and coronal sections of ~2-mm thickness were plated over ice. The striatum and hippocampus were bilaterally dissected using the stereotaxic coordinates derived from the Paxinos and Franklin (2012) mouse brain atlas. All protocols were approved by the Institutional Animal Care and Use Committee at the UPR-SOM and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2012) and AVMA Guidelines for the Euthanasia of Animals (2013).

Crude synaptosomal preparations

For the first experiment, the striatum and hippocampus of gp120 and WT mice (n=4/strain/region) were each homogenized in 1 ml of ice-cold buffer containing 0.32 M sucrose, 1 mM EDTA, and 4 mM Tris (pH 7.4) using a glass homogenizer (8–10 strokes; Kontes, CA). Homogenates were centrifuged at 1000×g for 10 min in a microcentrifuge at 4 °C (Sorvall 23R, Thermo Scientific). The resulting pellet (P1) was discarded and the supernatant (S1) was centrifuged at 10,000×g for 10 min at 4 °C. The resulting pellet (P2) constituted the crude synaptosomal fraction, which contains a rich mixture of neurons and glial cells (Dunkley et al. 2008). This fraction was suspended to a final volume of 1 ml with SEDH solution (0.32 M sucrose, 1 mM EDTA, 0.25 mM dithiothreitol (DTT), and 20 mM HEPES, pH 7.4) to give a protein concentration of ~0.5 mg/ml as determined by the Bradford/BSA Protein Assay (BioRad, CA). The total preparation time was approximately 1 h.

Glial plasmalemmal vesicles and synaptosomes

These glial and neuronal subcellular fractions allow for the direct comparison of glutamate uptake systems in preparations enriched with vesicles 'pinched off' from glial membranes (i.e., glial plasmalemmal vesicles (GPV)) or purified nerve endings (i.e., synaptosomes (SYN)). This approach has been extensively validated using morphological, immunohistochemical, and pharmacological measures (Nakamura et al. 1993; Hirst et al. 1998; Suchak et al. 2003; Dunkley et al. 2008). As such, the striatum of gp120 and WT mice (n=4/group) was homogenized in 1 ml of homogenization buffer and centrifuged at 1000×g for 10 min at 4 °C. The resulting S1 fraction (~0.5 ml) was carefully layered using a microsyringe pump (0.1 ml/min) onto a four-step discontinuous gradient

composed of 20, 10, 6, and 2 % Percoll (0.25 ml/gradient; GE Healthcare Biosciences) in SEDH solution. Each gradient was carefully layered the previous night using high G-force microcentrifuge tubes (VWR International, PA) and left to stand at 4 °C. The tubes were centrifuged at 33,500*g* × 5 min at 4 °C. The GPV fraction was carefully collected from the interfacial layer of the 2 and 6 % gradients, while synaptosomes were collected from the interface of the 10 and 20 % gradients, as described previously (e.g., Suchak et al. 2003). Each fraction was washed twice in 1 ml of ice-cold SEDH by centrifugation at 18,500*g* × 5 min at 4 °C. The GPV and synaptosomal fractions were resuspended to a final volume of 0.5 ml with SEDH to give a protein concentration of ~0.10 and 0.25 mg/ml, respectively (Bradford/BSA; Bio-Rad). The total preparation time was approximately 3.5 h.

Glutamate uptake assay

The uptake reaction was started by the addition of 25 μl of the corresponding tissue fraction in test tubes containing L-[^{3}H]-glutamate (100 nM; specific activity 49.0 Ci/mmol; Perkin Elmer, MA) and 0.1–100 μM of unlabeled glutamate (for saturation analysis) in 200 μl of uptake buffer containing (in mM) 140 NaCl, 5 KCl, 1.3 $CaCl_2$, 1 $MgCl_2$, 0.4 KH_2PO_4, 10 glucose, and 20 HEPES (pH 7.4) and incubated at 37 °C for 2 min. System x^-_c uptake was measured similarly except by replacing sodium chloride with equal concentrations of choline chloride. Under this condition, the system mediates the uptake of cystine along with tritiated glutamate in exchange of unlabeled intracellular glutamate, as described previously (e.g., Patel et al. 2004; Melendez et al. 2005). Hence, this system utilizes normally high intracellular levels of glutamate to drive the import of cystine (Patel et al. 2004). Uptake was terminated by rapid filtration with ice-cold sodium-free buffer onto a pre-wetted Whatman GF/B filter paper using a tissue harvester (Brandel, MD). Filters were dissolved overnight in scintillation fluid (EcoLite, MP Biomedicals, CA) and processed for scintillation counting (Beckman Instruments, USA).

Western blot analysis

Western blot analysis was performed on crude (mixed) synaptosomal preparations of the striatum and hippocampus (n=4–5/strain/region) to determine the expression of GLT-1 protein, which is the predominant glutamate transporter in the CNS (Danbolt 2001). Proteins were separated using 10 % SDS-PAGE and transferred electrophoretically from the gel onto a PVDF membrane (Bio-Rad, CA). Subsequently, the membrane was blocked in 5 % nonfat dry milk in Tris-buffered saline Tween 20 at room temperature and probed overnight at 4 °C with primary antibodies (Cell Signaling, MA) against rabbit GLT-1 (1:500) and mouse β-actin (1:1000), a ubiquitous cytoskeletal protein used as a loading (marker) control. Labeled proteins were detected with the use of a horseradish peroxidase-conjugated antirabbit secondary immunoglobulin G diluted to 1:5000. The Odyssey (LI-COR Biosciences, US) photoimage scanner was used to capture the infrared fluorescent signal from GLT-1 and β-actin on the same Western blot, without film, a darkroom, or any chemiluminescent substrates. The relative levels of proteins were quantified with the use of Odyssey software (Li-COR) and analyzed as the ratio of GLT-1/β-actin protein expression.

Statistical analyses

The maximal velocity (V_{max}) and the affinity (K_m) constants for systems X_{AG} and x_{c-} glutamate uptake were determined by nonlinear regression analysis using Prism (GraphPad software, CA) by applying the Michaelis Menten equation ($Y = V_{max} * X/(K_m + X)$) to the data. Significant differences in the estimated V_{max} and K_m values were determined by mixed-factorial analysis of variance (ANOVA) using the SPSS statistical software package (IBM, USA). For immunoblot analysis, differences in the ratio of GLT-1/β-actin protein expression were analyzed by simple one-way ANOVAs. In all cases, p values <0.05 were considered significant.

Results

We initially determined the kinetic properties of glutamate uptake of systems X_{AG} (sodium-dependent) and x_{c-} (sodium-independent) in crude (mixed) synaptosomes from the striatum and hippocampus of gp120 and WT mice. The kinetic constants V_{max} and K_m were used to distinguish between the number of glutamate transporters and affinity for glutamate, respectively. Figure 1 shows the saturation curves for systems X_{AG} and x_{c-} glutamate uptake in the striatum and hippocampus. Analysis of the striatum (Fig. 1a) revealed a significant reduction in the V_{max} of both systems X_{AG} and x_{c-} in gp120 mice compared to WT controls ($F_{1,6}$>6.75; p<0.05). There were no significant differences in the K_m for neither system X_{AG} nor x_{c-} in either strain ($F_{1,6}$<0.69; p>0.43), suggesting a reduction in the number of X_{AG} and x_{c-} glutamate transporters in the striatum of gp120 mice. Estimated V_{max} constants for systems X_{AG} and x_{c-} were (in pmol/mg protein/time) 115.9±19.1 and 15.7±1.3 for gp120 mice and 168.5±6.6 and 24.2±1.9 for WT, respectively. Estimated K_m constants for systems X_{AG} and x_{c-} were (in μM) 5.2±1.3 and 13.2±1.2 for gp120 mice and 4.3±0.6 and 11.5±1.5 for WT controls, respectively. Analysis of the kinetic parameters of glutamate uptake in the hippocampus (Fig. 1b) indicated no significant differences in neither the V_{max} nor K_m of systems X_{AG} and x_{c-} between gp120 and WT mice ($F_{1,6}$<0.37; p>0.56), suggesting that glutamate uptake systems in the hippocampus are less susceptible to HIV-1 gp120

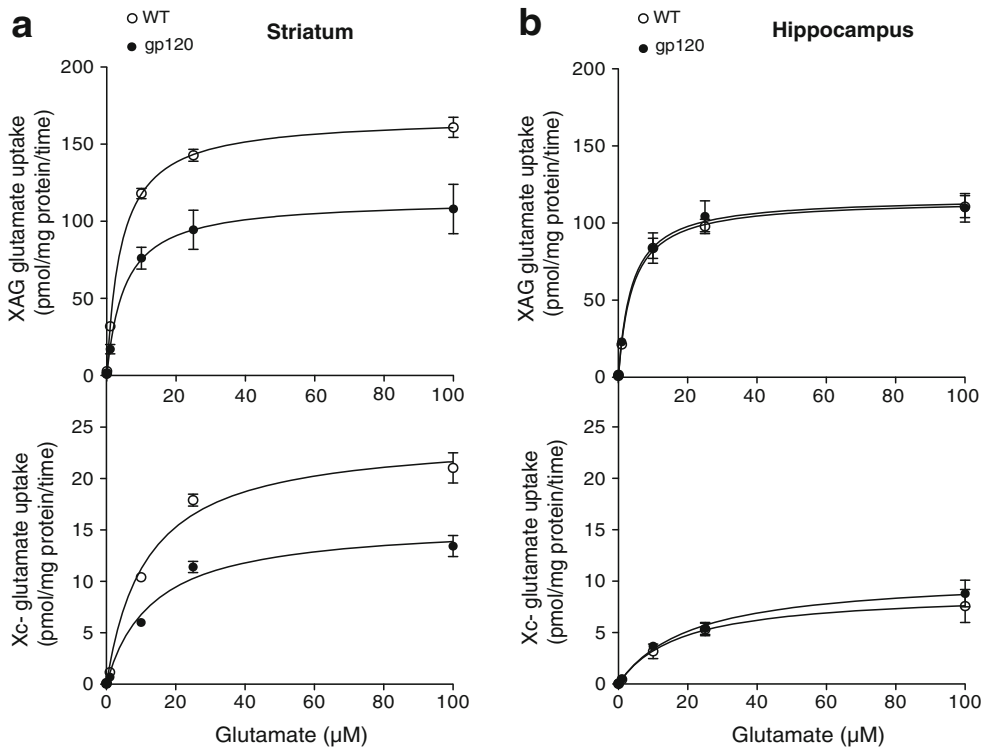

Fig. 1 System X_{AG} and x_{c-} glutamate uptake in crude (mixed) synaptosomes from the striatum and hippocampus of gp120 and wild-type (WT) mice. The maximal velocity of X_{AG} (i.e., sodium-dependent) and x_{c-} (i.e., sodium-independent) glutamate uptake (pmol/mg protein/time) was plotted as a function of the external unlabeled glutamate concentration. Saturation analysis was performed using 100 nM of L-[^{3}H]-glutamate in the absence or presence of unlabeled L-glutamate (0–100 μM) to give the final concentrations shown (*x-axis*). The maximal velocity (V_{max}) and the affinity (K_m) constants were determined by nonlinear regression analysis by applying the Michaelis Menten equation to the data. Significant differences in the estimated V_{max} and K_m values were determined by analysis of variance (ANOVA). A significant ($p<0.05$) reduction in the V_{max} of both systems X_{AG} and x_{c-} glutamate uptake was observed in the striatum (**a**) but not in the hippocampus (**b**) of gp120 mice compared to WT. There were no significant group differences in the K_m for glutamate in either glutamate uptake systems in the striatum and hippocampus. Data are expressed as mean±SEM (n=4/group)

exposure. Estimated V_{max} constants for X_{AG} and x_{c-} glutamate uptake were (in pmol/mg protein/time) 118.2±9.5 and 10.7±1.7 for gp120 mice and 116.3±6.2 and 9.1±1.9 for WT, respectively. Estimated K_m constants for systems X_{AG} and x_{c-} were (in μM) 4.2±0.7 and 21.1±2.5 for gp120 mice and 4.4±0.7 and 18.4±4.1 for WT controls, respectively.

In separate cohorts of mice, we then determined whether gp120 inhibition of striatal glutamate uptake was specific to neurons versus glial cells. To do so, we employed a subcellular fractionation method that gives rise to both purified synaptosomes (SYN) and glial plasmalemmal vesicles (GPV) from the same sample. Figure 2 shows the saturation curves for glutamate uptake systems X_{AG} and x_{c-} in GPV (Fig. 2a) and SYN (Fig. 2b) fractions from gp120 and WT mice. Analysis of the estimated V_{max} constants for systems X_{AG} and x_{c-} showed significant effects of strain ($F_{1,12}>32.8$; $p<0.01$) and fraction ($F_{1,12}>32.8$; $p<0.01$), but no strain × fraction interactions ($F_{1,12}<1.78$; $p>0.20$). Further analysis indicated a significant decrease in the V_{max} of X_{AG} and x_{c-} glutamate uptake in both GPV ($F_{1,6}>17.4$; $p<0.01$) and SYN ($F_{1,16}>9.61$; $p<0.05$) fractions from the striatum of gp120 mice relative to WT. Analysis of the estimated K_m values for systems X_{AG} and x_{c-} showed no significant effects of strain ($F_{1,6}<2.49$; $p>0.16$) or fraction ($F_{1,6}<2.49$; $p>0.16$), nor a strain × fraction interaction ($F_{1,6}<2.49$; $p>0.16$). The estimated V_{max} and K_m constants for X_{AG} and x_{c-} glutamate uptake in GPV and SYN fractions are illustrated in Fig. 3.

Summaries of the kinetic parameters for glutamate uptake by systems X_{AG} and x_{c-} in all subcellular fractions from the striatum are shown in Fig. 3. Notably, several key properties underlying X_{AG} and x_{c-} uptake were observed in both strains of mice. First, as expected, the V_{max} of system X_{AG} was significantly greater than system x_{c-} in all subcellular fractions ($p<0.01$), indicating that the majority of glutamate uptake is sodium-dependent. Second, the V_{max} of both systems X_{AG} and x_{c-} were significantly greater in mixed and GPV fractions compared to SYN ($p<0.05$), which is consistent with an elevated number of glutamate transporters in glial cells compared to neurons (Danbolt 2001). In final, the K_m constants for glutamate were significantly lower in system X_{AG} compared to x_{c-} ($p<0.05$), which is consistent with high-affinity glutamate uptake under sodium-dependent conditions in both glial and neuronal cells (Danbolt 2001).

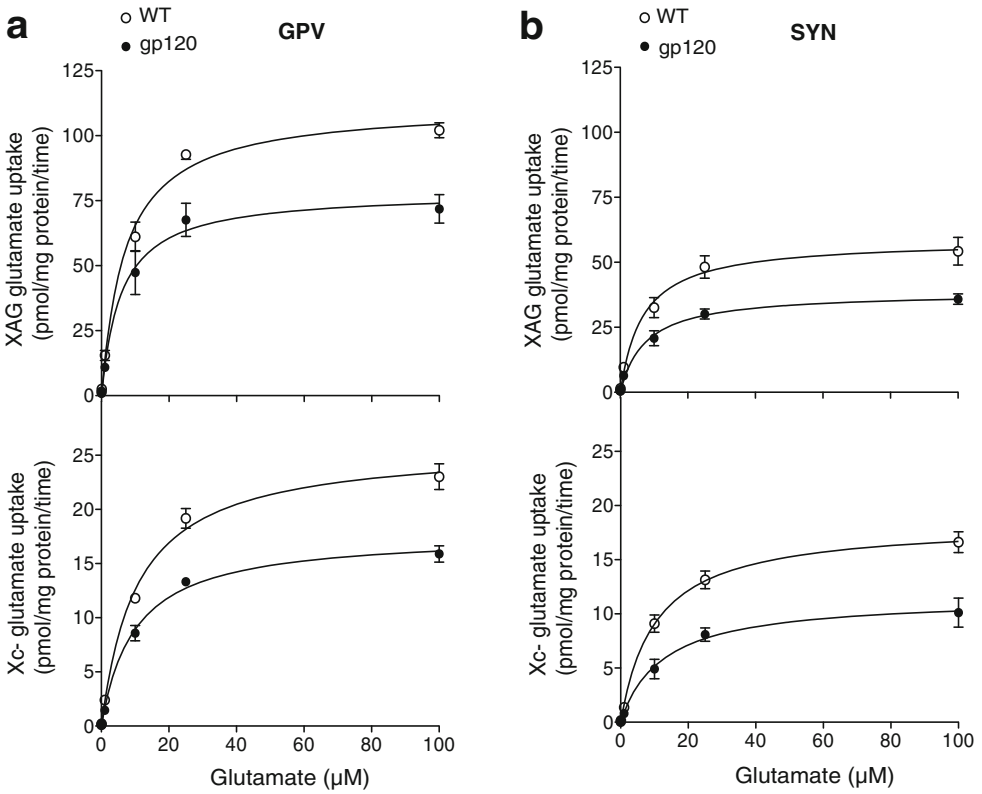

Fig. 2 Saturation analysis for system X_{AG} and x_{c-} glutamate uptake in glial and neuronal preparations from the striatum of gp120 and wild-type (WT) mice. These glial (i.e., glial plasmalemmal vesicles; GPV) and neuronal (i.e., synaptosomes; SYN) preparations are enriched in small vesicles pinched off from glial membranes and nerve endings, respectively. Analysis revealed a significant ($p<0.05$) reduction in the V_{max} of systems X_{AG} and x_{c-} in both **a** GPV and **b** SYN fractions from gp120 mice compared to WT. There were no significant group differences in the K_m (affinity) for X_{AG} or x_{c-} glutamate uptake in GPV and SYN. Data are expressed as mean ±SEM (n=4/preparation/group)

In final, we determined whether the observed gp120 inhibition of glutamate uptake correlates with the expression of GLT-1 protein, the primary glutamate transporter in CNS (Danbolt 2001). As illustrated in Fig. 4a, crude synaptosomal fractions from the striatum and hippocampus of gp120 and WT mice were subjected to Western blot analysis of GLT-1, standardized by the levels of β-actin. As shown in Fig. 4b, GLT-1 protein levels were significantly reduced (75.6±8 % of WT controls) in the striatum but not in the hippocampus of gp120 mice relative to WT controls ($F_{2,12}$=3.47; p=0.03), which is consistent with the kinetic profile observed in Fig. 3. Together, these findings indicate that the expression and function of GLT-1 are particularly impaired in the striatum of gp120 mice.

Discussion

The present study is the first to characterize the kinetic properties of glutamate uptake in HIV-1 gp120 transgenic mice, an

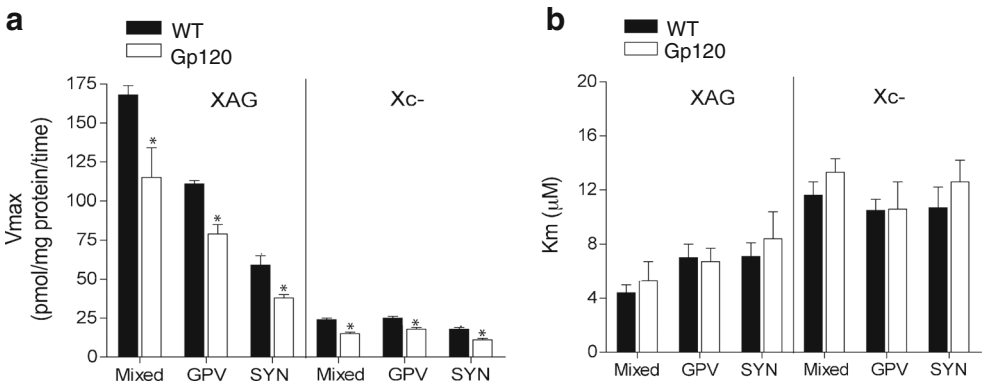

Fig. 3 Summary of the kinetic properties of system X_{AG} and x_{c-} glutamate uptake in the striatum of gp120 and WT mice. Summary of results demonstrating a significant deficit in the V_{max} both system X_{AG} and x_{c-} in neuronal and glial cells from the striatum of gp120 mice. Moreover, several key properties regarding the specificity of high-affinity and high-capacity glutamate uptake were revealed. **a** The V_{max} of system X_{AG} was significantly greater than x_{c-} in all striatal preparations (mixed, GPV, and SYN). Furthermore, the V_{max} of systems X_{AG} and x_{c-} were significantly greater in mixed and GPV compared to SYN preparations. **b** The K_m constants for glutamate were significantly lower in system X_{AG} relative to system x_{c-} ($p<0.05$) across all preparations. (*Asterisk*) $p<0.05$ compared to glial preparations (i.e., crude or GPV)

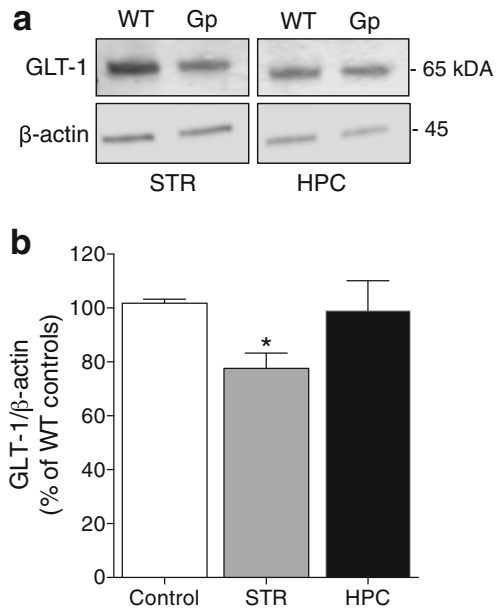

Fig. 4 Western blots of GLT-1 protein derived from crude synaptosomal preparations from the striatum (*STR*) and hippocampus (*HPC*) of gp120 (*GP*) and wild-type (*WT*) mice. **a** Representative immunoblots for GLT-1 and β-actin proteins yielded a band at approximately 65 and 45 kDa, respectively. **b**. Mean density of GLT-1 and β-actin immunoreactivity was expressed as the difference in ratio of GLT-1/β-actin protein expression and normalized to percent of WT controls. A significant ($p<0.05$) reduction in total protein levels of GLT-1 was observed in the STR but not in the HPC of gp120 mice compared to WT ($p<0.05$ one-way ANOVA). Data are expressed as mean±SEM ($n=5$/region/group)

established model of HIV-associated neurocognitive deficits. Glutamate transporters play an indispensable role in maintaining extracellular glutamate homeostasis, which is necessary for learning, memory, and cognitive processing. The results presented here revealed a significant reduction in the V_{max} of system X_{AG} (sodium-dependent) and system x_{c-} (sodium-independent) in both glial and neuronal preparations from the striatum of gp120 mice relative to WT controls. There were no significant group differences in the K_m for glutamate, suggesting that gp120 reduced the number of striatal glutamate transporters without affecting the affinity for glutamate. Consistent with the kinetic analysis, we also indicated a significant reduction in the total expression of GLT-1 protein in the striatum of gp120 mice, which is likely involved in the development or severity of HIV-associated neurocognitive deficits.

Several investigators have previously shown that HIV-1 or gp120 inhibits glutamate uptake in cultured glial cells (Dreyer and Lipton 1995; Vesce et al. 1997; Patton et al. 2000; Belmadani et al. 2001; Wang et al. 2003). It is also shown that gp120 alters the exchange of sodium and potassium in glial membranes (Benos et al. 1994; Holden et al. 1999), which typically leads to inhibition of glutamate uptake (Danbolt 2001). Interestingly, Wang et al. (2003) further indicated that gp120 inhibition of glutamate uptake was linked to a significant reduction in the expression of GLT-1 protein (Wang et al. 2003), the predominant glutamate transporter in the brain (Danbolt 2001). In the present study, we also show significant decreases in both glutamate uptake and GLT-1 expression in glial preparations from the striatum of gp120 mice. Taken together, these findings indicate that glial glutamate transporters are susceptible to gp120 exposure both in vitro and in vivo. The precise mechanisms, however, responsible for gp120-mediated downregulation of GLT-1 expression and function remain to be investigated.

Apart from glia, few studies if any have determined the impact of gp120 exposure on neuronal glutamate transporters. In our study, neuronal (i.e., SYN) glutamate uptake accounted for approximately 35 % of total glutamate uptake (Fig. 3), which is in agreement with previous results (Hirst et al. 1998). Studies suggest that the majority of glutamate uptake in neurons is mediated in part between GLT-1 and EAAC1 (excitatory amino acid carrier subtype-1) transporters (Danbolt 2001; Suchak et al. 2003). Here, similar to glial cells, we also show a significant deficit in neuronal glutamate uptake in the striatum of gp120 mice, and without altering the K_m for glutamate. Although EAAC1 was not measured for Western blot analysis, the possibility exists that gp120 may reduce both GLT-1 and EAAC1 protein expression in neuronal cells, which could lead to alterations in synaptic glutamate transmission and plasticity (Nieoullon et al. 2006; Waxman et al. 2007; Scimemi et al. 2009). Indeed, most neuronal glutamate transporters are localized primarily to dendritic spines, primary sites of synaptic plasticity that receive the majority of glutamatergic inputs (Rothstein et al. 1994; Danbolt 2001). Interestingly, increased activity of N-methyl-D-aspartate (NMDA) receptor function, which is necessary for the induction of synaptic plasticity, is often associated with greater cell surface expression of EAAC1 compared to GLT-1 (Nieoullon et al. 2006; Waxman et al. 2007; Scimemi et al. 2009).

Beyond sodium-dependent (X_{AG}) glutamate uptake, we also observed a significant deficit in the V_{max} of sodium-independent (x_{c-}) glutamate uptake in both glial and neuronal preparations from the striatum of gp120 mice and also without altering the K_m for glutamate. System x_{c-} acts as a glutamate antiporter that mediates the exchange of extracellular cystine for intracellular glutamate (Bridges et al. 2012). The import of cystine through system x_{c-} is critical to glutathione synthesis and oxidative protection in various cells both outside and inside the CNS (Murphy et al. 1989). In the CNS, the protein distribution of system x_{c-} shows the antiporter to be localized in both neurons and glial cells (Bridges et al. 2012). Notably, disturbances in glutathione homeostasis may contribute to impaired immunity, which may lead to the progression of several immunological diseases, including HIV. For instance, HIV-1 infection induces oxidative stress in glial cells and neurons, which is often correlated with the neurocognitive deficits observed in some HIV patients (Shi et al. 1998; Pocernich et al.

2004). Thus, gp120-inhibition of system x_{c-} may lead to major disruptions in glutathione synthesis and the emergence of excitotoxic neuropathology.

A major limitation to this study is the lack of data to demonstrate the impact of gp120 on oxidative stress. Nonetheless, it is worth noting that independent of oxidative stress, system x_{c-} plays a crucial role in providing a significant source of extracellular glutamate in the striatum (Baker et al. 2002), which is important for activating glutamate receptors and modulating synaptic plasticity. Thus, the possibility exists that gp120 plays an important role in altering extracellular glutamate homeostasis via disruption of glutamate uptake in the striatum in addition to promoting neurotoxicity. In any case, understanding the role of system x_{c-} and other mechanisms that lead to alterations in glutamate and glutathione homeostasis in HIV-1 infection may advance the development of new therapeutic strategies to prevent synaptodendritic injury and improve cognitive function in HIV patients.

In the present study, it was interesting to observe that the expression and function of glutamate transporters were unaltered in the hippocampus of gp120 mice. This suggests brain region-specific alterations by gp120, potentially similar to those found in the brains of neurocognitive-impaired, HIV-infected humans (Moore et al. 2006). Indeed, there is evidence suggesting that the striatum (made up of the putamen and caudate nucleus) shows one of the highest burdens of HIV proteins and viral RNA following infection in the brain (Wiley et al. 1998). It is also possible that the perturbations observed in the striatum, but in not the hippocampus may reflect disturbances in the balance between synaptodendritic injury and repair. For instance, virus- and host-derived toxic factors that are active during HIV infection may be counteracted in the hippocampus by host trophic factor production and neuronal plasticity (Ellis et al. 2007). Future studies will be necessary to address the functional and brain-region specificity of HIV-1 gp120 on glutamate uptake systems.

To conclude, the results of the present study suggest that restoring glutamate uptake systems in the striatum may be a potential mechanism for restoring memory and cognitive performance in HIV-infected individuals. Research to this end will be amenable to new glutamate- and neuroplasticity-based pharmacotherapies for the treatment of HIV-associated neurocognitive disorders.

Acknowledgments All authors approved the final version of this article. The authors would like to thank L. Perez for his excellent technical assistance. This work was supported in part by National Institutes of Health Grants SNRP-U54N54301, 2R25GM061151 and NIGMS 1P20GM103642.

Compliance with ethical standards All protocols were approved by the Institutional Animal Care and Use Committee at the UPR-SOM and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2012) and AVMA Guidelines for the Euthanasia of Animals (2013).

Conflict of interest The authors declare that there is no conflict of interests.

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