Journal of NeuroVirology, 15: 449–457, 2009

ISSN 1355-0284 print / 1538-2443 online
DOI: 10.3109/13550280903350598

informa
healthcare

Short communication

GCP II inhibition rescues neurons from gp120IIIB-induced neurotoxicity

Ajit G Thomas,[1] Amos Bodner,[2] Ghanashyam Ghadge,[3] Raymond P Roos,[3] and Barbara S. Slusher[1]

[1]Brain Science Institute, NeuroTranslational Program, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; [2]Department of Neurobiology, Pharmacology, and Physiology; and [3]Department of Neurology, University of Chicago Medical Center, Chicago, Illinois, USA

Excessive glutamate neurotransmission has been implicated in neuronal injury in many disorders of the central nervous system (CNS), including human immunodeficiency virus (HIV)-associated dementia. Gp120IIIB is a strain of a HIV glycoprotein with specificity for the CXCR4 receptor that induces neuronal apoptosis in *in vitro* models of acquired immunodeficiency syndrome (AIDS)-induced neurodegeneration. Since the catabolism of the neuropeptide *N*-acetylaspartylglutamate (NAAG) by glutamate carboxypeptidase (GCP) II increases cellular glutamate, an event associated with excitotoxicity, we hypothesized that inhibition of GCP II may prevent gp120IIIB-induced cell death. Furthermore, through GCP II inhibition, increased NAAG may be neuroprotective via its agonist effects at the $mGlu_3$ receptor. To ascertain the therapeutic potential of GCP II inhibitors, embryonic day 17 hippocampal cultures were exposed to gp120IIIB in the presence of a potent and highly selective GCP II inhibitor, 2-(phosphonomethyl)-pentanedioic acid (2-PMPA). 2-PMPA was found to abrogate gp120IIIB-induced toxicity in a dose-dependent manner. Additionally, 2-PMPA was neuroprotective when applied up to 2 h after the application of gp120IIIB. The abrogation of apoptosis by 2-PMPA was reversed with administration of $mGlu_3$ receptor antagonists and with antibodies to transforming growth factor (TGF)-β. Further, consistent with the localization of GCP II, 2-PMPA failed to provide neuroprotection in the absence of glia. GCP II activity and its inhibition by 2-PMPA were confirmed in the hippocampal cultures using radiolabeled NAAG and high-performance liquid chromatography (HPLC) analysis. Taken together, these data suggest that GCP II is involved in mediating gp120-induced apoptosis in hippocampal neurons and GCP II inhibitors may have potential in the treatment of neuronal injury related to AIDS. *Journal of NeuroVirology* (2010) **15**, 449–457.

Keywords: GCP II; glutamate; HIV-associated dementia; metabotropic glutamate receptors; NAAG; TGF-β

Address correspondence to: Barbara S. Slusher, PhD, Brain Science Institute, Johns Hopkins University School of Medicine, 855 N. Wolfe Street, Baltimore, MD 21205, USA. E-mail: bslusher@jhmi.edu

Received 01 May 2009; revised 19 August 2009; accepted 14 September 2009.

Introduction

Acquired immunodeficiency syndrome (AIDS) is a disease that affects both the immune and nervous systems. Infection of the nervous system by the human immunodeficiency virus (HIV)-1 leads to a spectrum of cognitive and motor problems, including dementia, myelopathy, and peripheral neuropathy (Sacktor *et al*, 2001). Although HIV-1 is not thought to replicate in neurons (Kaul *et al*, 2001), the overexpression of the HIV-1 coat glycoprotein, gp120, has been shown to produce apoptotic neuronal death both *in vitro* and *in vivo* (Lipton, 1994; Meucci *et al*, 1998). The release of neurotoxic agents from brain macrophages and microglia has been suggested as a possible mechanism in gp120-induced cell death (Kaul *et al*, 2001). Additionally, gp120 was shown to affect the astrocytic control of extracellular glutamate by both inhibiting the uptake and stimulating the release of glutamate (Vesce *et al*, 1997). That excessive extracellular glutamate neurotransmission is central to neuronal injury is well documented. Extensive research has focused on postsynaptic glutamate receptor antagonism as a strategy to attenuate the pathological consequence of this excess (Lipton, 1998). Here, we describe a novel strategy for therapeutic intervention based on the inhibition of glutamate carboxypeptidase (GCP) II.

N-acetylaspartylglutamate (NAAG) has been implicated as a potential storage form of synaptic glutamate (Neale *et al*, 2005). The synaptically localized neuropeptidase GCP II (also termed *N*-acetylated-α-linked acidic dipeptidase, NAALADase; EC3.4.17.21), present on the external surface of astrocytic cells (Berger *et al*, 1999; Cassidy and Neale, 1993), hydrolyzes NAAG to free glutamate and *N*-acetyl-aspartate (NAA) both *in vitro* (Robinson *et al*, 1987) and *in vivo* (Stauch *et al*, 1989). Since the catabolism of NAAG increases cellular glutamate, a neurotransmitter associated with apoptosis, we hypothesized that inhibition of GCP II could reduce extracellular glutamate levels and provide a new strategy targeting gp120-induced neuronal apoptosis and perhaps consequently, AIDS-related neurotoxicity. Additionally, we hypothesized that GCP II inhibition could protect against gp120 toxicity via resultant increases in NAAG. NAAG is known to act as an agonist at the group II metabotropic glutamate receptors, specifically at the $mGlu_3$ receptor (Wroblewska *et al*, 1997), the effects of which have previously been shown to be neuroprotective (Bruno *et al*, 1998b; Thomas *et al*, 2000). Several mechanisms are thought to underlie the neuroprotection afforded by $mGlu_3$ receptor agonists, including reduced glutamate release through presynaptic receptor (Sanchez-Prieto *et al*, 1996), inhibition of cyclic adenosine monophosphate (cAMP) formation (Buisson and Choi, 1995), and the release of transforming growth factor (TGF)-β (Bruno *et al*, 1998a, 1998b).

In 1996, the first potent and selective GCP II inhibitor, termed 2-(phosphonomethyl)-pentanedioic acid (2-PMPA), was described (Jackson *et al*, 1996). In this report, we examined the therapeutic potential of 2-PMPA in hippocampal cultures exposed to gp120IIIB, a model of AIDS-related neuronal injury.

Results and discussion

2-PMPA rescues hippocampal neurons from gp120IIIB neurotoxicity in a dose- and time-dependent manner

Significant apoptosis occurred in hippocampal neurons exposed to gp120IIIB, at 200 pM (Figure 1A; data normalized to percent apoptosis in control and gp120IIIB-treated cells. Percentage of apoptotic cells in control and gp120III-treated were 22.3 ± 1.9 and 42.9 ± 2.1, respectively; $P < .001$). When 2-PMPA was applied coincident with the administration of the glycoprotein, 2-PMPA protected hippocampal neurons from gp120IIIB-induced apoptosis in a concentration-dependent manner (Figure 1A), with maximal effects between 1 and 10 μM. Time-dependence of neuroprotection was measured by applying 2-PMPA 10 μM at varying times after the initial application of gp120IIIB. The efficacy of 2-PMPA in preventing gp120IIIB-induced apoptosis was lost if delayed for greater than 2 h after insult (Figure 1B). Although the efficacious concentration of 2-PMPA is higher than its *in vitro* inhibitory constant with the purified enzyme (Jackson *et al*, 1996), these data are consistent with other reports, wherein 2-PMPA was shown to be neuroprotective in *in vitro* models of hypoxia (Tortella *et al*, 2000; Slusher *et al*, 1999; Thomas *et al*, 2001). Furthermore, this concentration of 2-PMPA was previously reported to be inactive in more than 100 different receptor, transporter, ion channel, and enzyme assays, including several glutamatergic assays involving *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and glutamate transporters (Slusher *et al*, 1999).

2-PMPA–mediated neuroprotection is abrogated by $mGlu_3$ receptor antagonists

Previous reports have shown that inhibition of GCP II is neuroprotective through a dual mechanism of action: via the reduction of extracellular glutamate and via the elevation of NAAG (Slusher *et al*, 1999; Thomas *et al*, 2000). NAAG functions as an agonist at the $mGlu_3$ receptors (Wroblewska *et al*, 1997). To examine whether the neuroprotection afforded by GCP II inhibition against gp120IIIB-induced apoptosis was mediated by $mGlu_3$ receptors, cultures were cotreated with 2-PMPA, gp120IIIB, and either LY341495 or (2***S***)-α-ethylglutamic acid (EGLU),

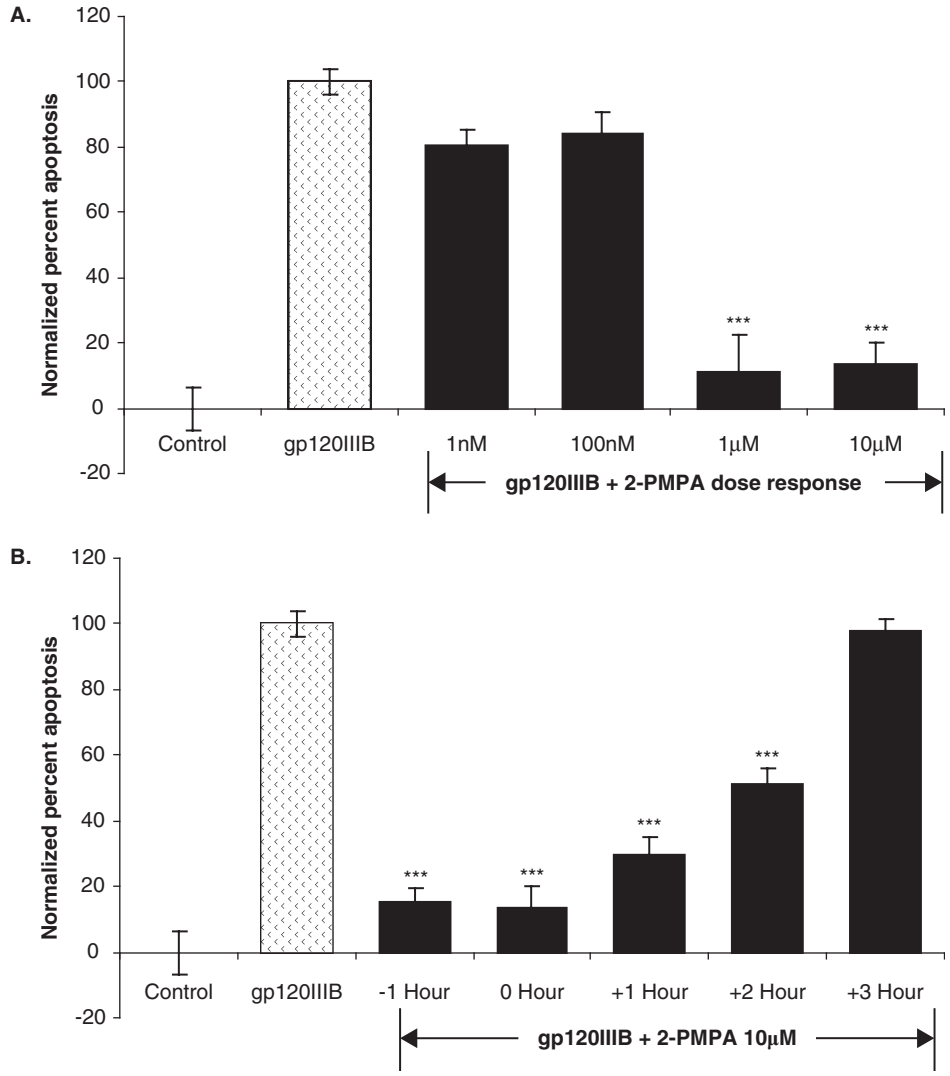

Figure 1 Concentration- and time-dependent neuroprotective effects of 2-PMPA. **(A)** Hippocampal neurons were coexposed to gp120IIIB (200 pM) and different concentrations of 2-PMPA and assessed for apoptosis after 4 days. 2-PMPA dramatically protected against gp120IIIB-induced apoptosis, with significant effects at the 1 to 10 μM dose. Data were normalized to control and gp120IIIB treatments; percentage of apoptotic neurons in control and gp120IIIB-treated dishes were 22.3 ± 1.9 and 42.9 ± 2.1, respectively. Data are the average of six independent experiments and asterisks indicate statistically significant differences from the gp120IIIB-treated group; ***P <.001. **(B)** 2-PMPA (10 μM) was applied to hippocampal neurons at various time points relative to the application of gp120IIIB, as indicated. 2-PMPA was neuroprotective when applied up to 2 h after the application of gp120IIIB. The efficacy was lost if the application of 2-PMPA was delayed for 3 h from the time of insult. Data are the average of four to six independent experiments and asterisks indicate statistically significant differences from the gp120IIIB-treated group; ***P <.001.

specific antagonists at the $mGlu_3$ receptor. While 2-PMPA 10 μM protected hippocampal neurons from gp120IIIB toxicity, cultures cotreated with either LY341495 or EGLU could not be rescued with 2-PMPA. The specificity of the $mGlu_3$ receptor in the mechanism of action of 2-PMPA was further shown by parallel cultures in which (***R,S***)-1-aminoindan-1,5-dicarboxylic acid (AIDA), (***R,S***)-α-methylserine-*O*-phosphate (MSOP), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), inhibitors of group I and group III mGlu receptors and AMPA receptors, respectively, did not alter the neuroprotection afforded by 2-PMPA (Figure 2). These data suggest that at least part of the neuroprotection is due to increased NAAG and $mGlu_3$ receptor activity.

2-PMPA–mediated neuroprotection requires TGF-β

Several mechanisms are thought to underlie the neuroprotection provided by $mGlu_3$ receptor agonists, including reduced glutamate release via presynaptic receptor activation and increased neuroprotective growth factor release via glial receptor activation (Neale *et al*, 2005). NAAG is an $mGlu_3$ receptor selective agonist with an EC_{50} of 65 μM (Wroblewska *et al*, 1997). Activation of glial $mGlu_3$ receptors inhibits the formation of cAMP and causes the activation of mitogen-activated protein

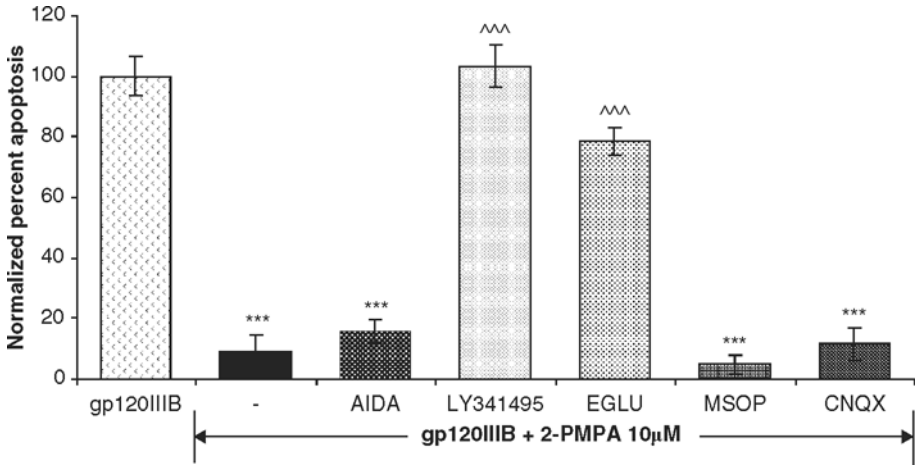

Figure 2 2-PMPA rescue of hippocampal neurons from gp120IIIB-induced apoptosis is mediated by group II mGluRs. 2-PMPA (10 μM) completely abrogated the neurotoxicity of gp120IIIB (200 pM). Rescue by 2-PMPA was significantly reduced by the application of group II metabotropic glutamate receptor antagonists, LY341495 and EGLU. AIDA, MSOP, and CNQX, antagonists at the group I, group III metabotropic glutamate receptors and AMPA receptors, respectively, did not alter the neuroprotection afforded by 2-PMPA. Concentration of antagonists in these experiments was 100 μM. Data are the average of four to six independent experiments. Asterisks and circumflex accents indicate statistical significance against gp120IIIB and gp120IIIB/2-PMPA, respectively; ***P <.001, ^^^P <.001.

kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways (Ferraguti *et al*, 1999; Genazzani *et al*, 1994; Pin and Duvoisin, 1995; Winder and Conn, 1993); the latter effects induce the *de novo* synthesis and release of TGF-β (Bruno *et al*, 1997, 1998a).

To examine the role of TGF-β in the neuroprotection mediated by 2-PMPA, two different approaches were used: (i) a direct method, wherein TGF-β (5 ng/ml) was exogenously applied to hippocampal neurons treated with gp120IIIB; and (ii) an indirect method, wherein a nonselective neutralizing antibody to TGF-β (10 μg/ml) was exogenously applied to hippocampal neurons cotreated with 2-PMPA (10 μM) and gp120IIIB.

That anti-TGF-β antibodies reversed the neuroprotection provided by 2-PMPA (Figure 3) suggests a neuroprotective role for TGF-β in GCP-II–mediated neuroprotective mechanisms in our paradigm of HIV-associated neuronal injury. In fact, selective activation of glial $mGlu_3$ receptors by NAAG has been shown to be neuroprotective via the release of TGF-β (Bruno *et al*, 1998a, 1998b). These results are consonant with our own previous reports demonstrating the neuroprotective effects of GCP II inhibition via the elaboration of TGF-β (Thomas *et al*,

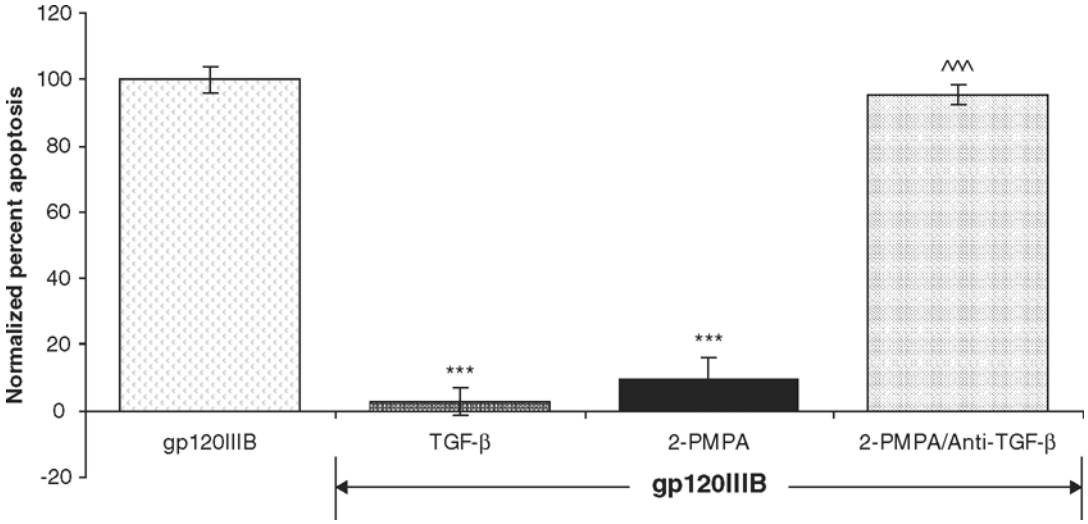

Figure 3 2-PMPA mediates the rescue of hippocampal neurons exposed to gp120IIIB by TGF-β. TGF-β (5 ng/ml) protected against gp120IIIB (200 pM) toxicity. Additionally, the neuroprotection afforded by 2-PMPA (10 μM) against gp120IIIB-induced apoptosis was reversed by the addition of a nonselective neutralizing antibody to TGF-β at 10 μg/ml. Data are an average of two to four independent experiments. Asterisks and circumflex accents indicate statistical significance against gp120IIIB and gp120IIIB/2-PMPA, respectively; ***P <.001, ^^^P <.001.

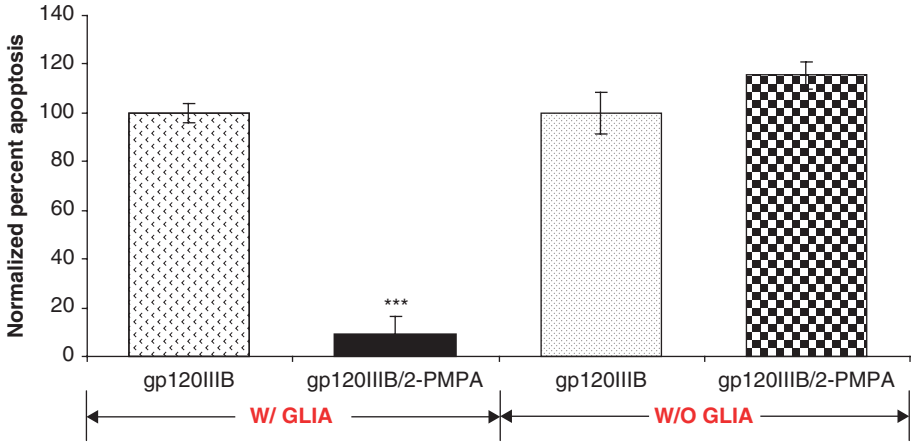

Figure 4 Efficacy of GCP II inhibition requires glia. Hippocampal neurons were treated with gp120IIIB (200 pM) and 2-PMPA (10 μM) in the presence and absence of glia. 2-PMPA failed to rescue hippocampal neurons from gp120IIIB-induced apoptosis in the absence of glia. Data are an average of two to four independent experiments and asterisks indicate statistical significance against the gp120IIIB-treated group in the presence of glia; ***p <.001.

2001). Furthermore, exogenous application of the cytokine has also been shown to be neuroprotective in several neuronal injury models, including gp120-induced neurotoxocity (Flanders *et al*, 1998; Meucci and Miller, 1996). Application of the cytokine by itself abrogated gp120IIIB-induced apoptosis (Figure 3).

2-PMPA–mediated neuroprotection requires the presence of glia

Hippocampal neurons were grown facing the glial feeder layer for the first 6 days of culture and then transferred to dishes without glia but containing the conditioned media with gp120IIIB and 2-PMPA on day *in vitro* 7 (DIV7). Cell survival was evaluated ~36 h after the addition of the glycoprotein. Gp120IIIB caused apoptosis of hippocampal neurons even in the absence of glia, providing evidence for the direct neurotoxic effects of the glycoprotein (Figure 4). Further, 2-PMPA provided no rescue against the neurotoxicity of the glycoprotein, thereby demonstrating the requirement for glia in the neuroprotection afforded by 2-PMPA (Figure 4). This loss of 2-PMPA–mediated neuroprotection in the absence of glia is consistent with the expression of GCP II on predominantly astrocytic cells (Berger *et al*, 1999; Cassidy and Neale, 1993).

2-PMPA inhibits GCP II activity in hippocampal cultures

Radiolabeled NAAG was used to examine the activity of GCP II in our model of neurotoxicity. High-performance liquid chromatography (HPLC) analysis of radiolabeled NAAG demonstrated a 55% conversion of the [^{3}H]-NAAG to [^{3}H]-glutamate (Table 1), confirming GCP II activity in our hippocampal neurons/astocytic coculture system. Additionally, as established previously (Slusher *et al*, 1999), treatment of the same cultures with 2-PMPA (10 μM) decreased [^{3}H]-glutamate levels and increased [^{3}H]-NAAG levels (Table 1, Figure 5), confirming 2-PMPA's mechanism of inhibition. That [^{3}H]-glutamate levels in the control condition were the same as that in the gp120IIIB-treated condition (Table 1) suggests that gp120IIIB did not induce any direct effects on GCP II activity.

Table 1 Confirmation of GCP II activity and 2-PMPA inhibition in the gp120IIIB-induced neurotoxicity model using [^{3}H]-NAAG

	%NAAG	% (Glutamate and metabolites)
Control	45 ± 13	55 ± 13
2-PMPA	91 ± 8	9 ± 8
gp120IIIB	44 ± 15	55 ± 15
gp120IIIB/2-PMPA	90 ± 10	9 ± 9

Note. Glutamate-labeled [^{3}H]-NAAG (2 μCi/4 ml medium) was added to hippocampal cultures grown in the presence of glia under various experimental conditions. After 4 days of continuous exposure, 1-ml aliquots of the culture medium were analyzed for [^{3}H]-NAAG and [^{3}H]-glutamate on a radiomatic detector using a strong anion-exchange column. Data are presented as a percent of the radioactivity measured under control conditions. Table shows the effect of 2-PMPA on the hydrolysis of [^{3}H]-NAAG.

In summary, we show 2-PMPA, a potent inhibitor of the astrocytic membrane peptidase GCP II, to be an effective inhibitor of neuronal apoptosis induced by gp120IIIB. It is suggested that this neuroprotection is mediated by increased NAAG and its consequent effects at $mGlu_3$ receptors with the release of TGF-β.

In conclusion, we demonstrate for the first time that GCP II inhibition abrogates neuronal apoptosis due to gp120IIIB, suggesting the potential clinical utility of this approach for inhibiting the progression of AIDS dementia. GCP II inhibition is an effective neuroprotective strategy given that HIV-1 neurotoxicity involves glutamate excitotoxicity (Meucci *et al*, 1998). In fact, *N*-methyl-D-aspartate

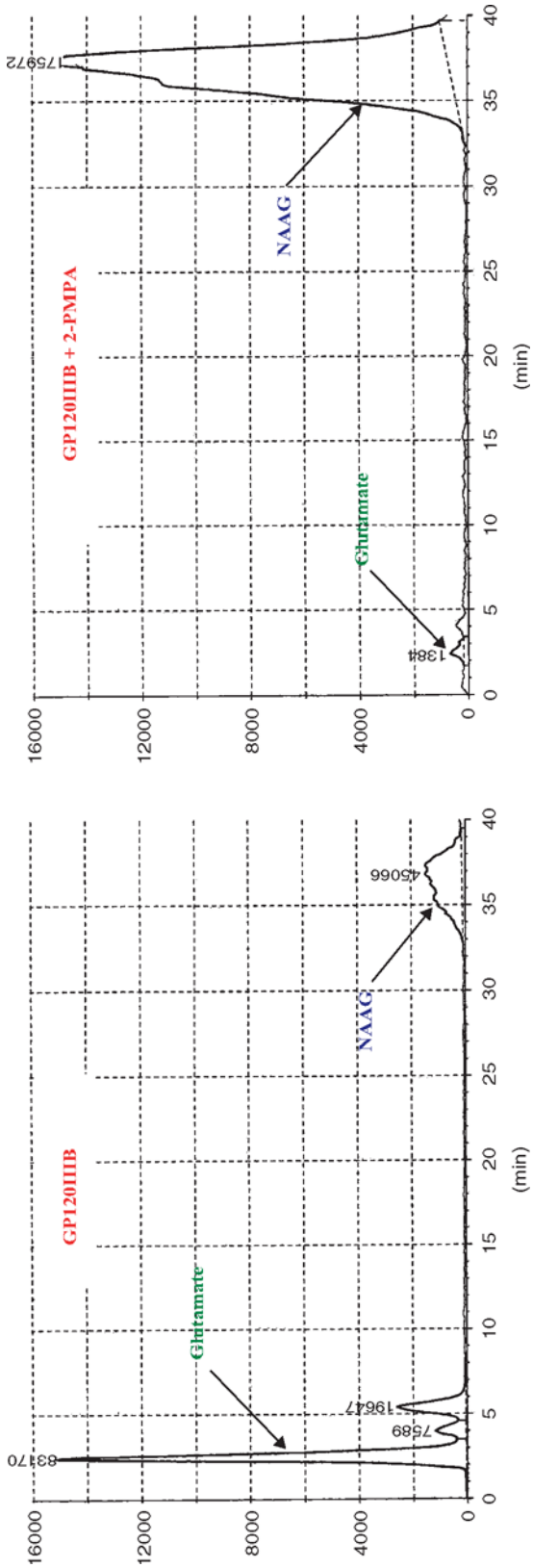

Figure 5 Representative chromatograms from medium analysis of [^{3}H]-NAAG in the presence and absence of 2-PMPA. Glutamate-labeled [^{3}H]-NAAG (2 μCi/4 ml medium) was added to hippocampal cultures grown in the presence of glia. After 4 days of continuous exposure, 1-ml aliquots of the culture medium were analyzed for [^{3}H]-NAAG and [^{3}H]-glutamate on a radiomatic detector using a strong anion-exchange column. Left panel indicates significant hydrolysis of [^{3}H]-NAAG in the absence of 2-PMPA. Right panel indicates near complete inhibition of the hydrolysis of [^{3}H]-NAAG in the presence of 2-PMPA.

(NMDA) inhibition has been shown to rescue neurons from gp120 neurotoxicity (Lipton *et al*, 1991; Sindou *et al*, 1994). Additionally, GCP II inhibition is an effective approach given that TGF-β abrogates the neuronal death associated with the glycoprotein (Meucci and Miller, 1996). Animal studies aimed at further elucidating the potential role of GCP II inhibitors in the treatment of HIV-associated dementia are being planned.

Materials and methods

Materials
2-PMPA was synthesized by SRI International (Menlo Park, CA). EGLU and LY341495 (group II $mGlu_3$ receptor antagonists, AIDA; a group I mGlu receptor antagonist, MSOP; a group III mGlu receptor antagonist and CNQX; a competitive non-NMDA receptor antagonist) were obtained from Tocris-Cookson Inc. (Ellisville, MO). TGF-β and antibody to TGF-β (anti-TGF-β) were purchased from Sigma (St. Louis, MO). Radiolabeled NAAG was obtained from Perkin Elmer Life Science (Boston, MA). Hoechst 33342 was obtained from Molecular Probes/Invitrogen (Eugene, OR). Gp120IIIB was kindly supplied by the National Institute of Allergy and Infectious Diseases (NIAID) AIDS Reagent Program (National Institutes of Health, Bethesda, MD).

Neuronal cultures and assessment of gp120IIIB-induced neuronal death
Primary hippocampal neurons were obtained from the hippocampi of rat embryos at days 17 to 18 of gestation, as previously described (Abele *et al*, 1990; Meucci and Miller, 1996). The neurons were plated onto 15-mm glass coverslips ($1–2 \times 10^4$ cells/cm^2) and cultured in defined Dulbecco's modified Eagle's medium (DMEM) containing N-2 supplement and 1 mg/ml ovalbumin. A feeder layer of astrocytes facing the neurons was used to support their growth and differentiation. Astroglia contamination of neuronal cultures was lower than 5%, as assessed by glial fibrillary acidic protein immunostaining. Microglial contamination of neuronal cultures, as assessed by ionized calcium-binding adaptor molecule 1 (Iab1) immunostaining, was negligible (1%), whereas microglia were <3% in the glial feeder layer. Virtually pure neuronal cultures were obtained when cells were plated at very low density. Cytosine arabinofuranoside (5 μM) was added to the cultures 24 h after plating to halt nonneuronal cell proliferation.

Neurons were treated with gp120IIIB (200 pM), the strain of the glycoprotein with specificity for the CXCR4 receptor, in the presence of glia. Treatment was started on the seventh day of culture, with or without 2-PMPA, and carried on for 4 days (Meucci and Miller, 1996). For experiments in the absence of glia, neurons were grown facing the glial feeder layer for the first 6 days of culture and then moved to dishes containing their conditioned medium when experimental treatments were started on DIV7; cell survival was evaluated ~36 h after the addition of gp120IIIB 200 pM. Although there was a higher background of cell death under these conditions because of glial deprivation, gp120IIIB neurotoxicity was still evident.

At the end of the experiment, cells were fixed and assessed for apoptosis. Hoechst 33342 (5 μg/ml) was used to evaluate differences between normal and apoptotic neurons (Meucci and Miller, 1996). Alive and apoptotic neurons in each microscopic field were counted and the percentage of apoptotic neurons was calculated; cells possessing condensed nuclei or fragmented chromatin were scored as apoptotic. As described previously (Bodner *et al*, 2002), for each condition, data were obtained from five coverslips of neurons grown in the same culture dish and exposed to the same conditions. Four random fields were assessed on each coverslip for counts of apoptotic neurons. A total of 70 to 500 neurons were counted and the final data are an average of two to six experiments. Data were then normalized within each experiment as a percent of gp120IIIB and control: ((condition − control) × 100). Student's two-population t-test was used for statistical analysis.

HPLC analysis of [^{3}H]-NAAG and [^{3}H]-glutamate
Following the addition of reagents as indicated above, radiolabeled NAAG (2 μCi/4 ml medium) was added to hippocampal cultures grown in the presence of glia on DIV7. After 4 days of continuous exposure, 1-ml aliquots of the culture medium were lyophilized at room temperature for 18 h and then reconstituted in 250 μl methanol:water (80:20). Undissolved particles were removed upon ultracentrifugation at 500,000 × *g* for 30 min at 4°C. Fifty microliter of each of the samples was analyzed for [^{3}H]-NAAG and [^{3}H]-glutamate on a Packard's Radiomatic detector using a strong anion-exchange column (ES industries) with a 0.1 M KH_2PO_4/0.1 M KCl (pH 4.5) mobile phase at 1.5 ml/min flow rate. [^{3}H]-NAAG and [^{3}H]-glutamate standards were injected (20 μl) at the beginning and at the end of the runs to insure the reliability/reproducibility of the column.

Acknowledgment

This article was submitted in memory of Dr. Amos Bodner, MD.

The authors wish to acknowledge the expert technical assistance of Christopher Mauer, and the proficient assistance of Deborah Stoit.

Declaration of interest: This work was supported by grants from the NIH DA012121, DA13141, MH40165, NS33826, DK44840, and NS21442 to Richard J. Miller. Amos Bodner was partially supported by a fellowship for careers in clinical pharmacology from the Pharmaceutical Research and Manufacturers of America Foundation, as well as a gift from Guilford Pharmaceuticals, Inc. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Abele AE, Scholz KP, Scholz WK, Miller RJ (1990). Excitotoxicity induced by enhanced excitatory neurotransmission in cultured hippocampal pyramidal neurons. *Neuron* **4:** 413–419.

Berger UV, Luthi-Carter R, Passani LA, Elkabes S, Black I, Konradi C, Coyle JT (1999). Glutamate carboxypeptidase II is expressed by astrocytes in the adult rat nervous system. *J Comp Neurol* **415:** 52–64.

Bodner A, Maroney AC, Finn JP, Ghadge G, Roos R, Miller RJ (2002). Mixed lineage kinase 3 mediates gp120IIIB-induced neurotoxicity. *J Neurochem* **82:** 1424–1434.

Bruno V, Battaglia G, Casabona G, Copani A, Caciagli F, Nicoletti F (1998a). Neuroprotection by glial metabotropic glutamate receptors is mediated by transforming growth factor-beta. *J Neurosci* **18:** 9594–9600.

Bruno V, Sureda FX, Storto M, Casabona G, Caruso A, Knopfel T, Kuhn R, Nicoletti F (1997). The neuroprotective activity of group-II metabotropic glutamate receptors requires new protein synthesis and involves a glial-neuronal signaling. *J Neurosci* **17:** 1891–1897.

Bruno V, Wroblewska B, Wroblewski JT, Fiore L, Nicoletti F (1998b). Neuroprotective activity of N-acetylaspartylglutamate in cultured cortical cells. *Neuroscience* **85:** 751–757.

Buisson A, Choi DW (1995). The inhibitory mGluR agonist, S-4-carboxy-3-hydroxy-phenylglycine selectively attenuates NMDA neurotoxicity and oxygen-glucose deprivation-induced neuronal death. *Neuropharmacology* **34:** 1081–1087.

Cassidy M, Neale JH (1993). *N*-acetylaspartylglutamate catabolism is achieved by an enzyme on the cell surface of neurons and glia. *Neuropeptides* **24:** 271–278.

Ferraguti F, Baldani-Guerra B, Corsi M, Nakanishi S, Corti C (1999). Activation of the extracellular signal-regulated kinase 2 by metabotropic glutamate receptors. *Eur J Neurosci* **11:** 2073–2082.

Flanders KC, Ren RF, Lippa CF (1998). Transforming growth factor-betas in neurodegenerative disease. *Prog Neurobiol* **54:** 71–85.

Genazzani AA, L'Episcopo MR, Casabona G, Shinozaki H, Nicoletti F (1994). (2S,1′R,2′R,3′R)-2-(2,3-dicarboxycyclopropyl) glycine positively modulates metabotropic glutamate receptors coupled to polyphosphoinositide hydrolysis in rat hippocampal slices. *Brain Res* **659:** 10–16.

Jackson PF, Cole DC, Slusher BS, Stetz SL, Ross LE, Donzanti BA, Trainor DA (1996). Design, synthesis, and biological activity of a potent inhibitor of the neuropeptidase *N*-acetylated alpha-linked acidic dipeptidase. *J Med Chem* **39:** 619–22.

Kaul M, Garden GA, Lipton SA (2001). Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature* **410:** 988–994.

Lipton SA (1994). HIV-related neuronal injury. Potential therapeutic intervention with calcium channel antagonists and NMDA antagonists. *Mol Neurobiol* **8:** 181–196.

Lipton SA (1998). Neuronal injury associated with HIV-1: approaches to treatment. *Annu Rev Pharmacol Toxicol* **38:** 159–177.

Lipton SA, Sucher NJ, Kaiser PK, Dreyer EB (1991). Synergistic effects of HIV coat protein and NMDA receptor-mediated neurotoxicity. *Neuron* **7:** 111–118.

Meucci O, Fatatis A, Simen AA, Bushell TJ, Gray PW, Miller RJ (1998). Chemokines regulate hippocampal neuronal signaling and gp120 neurotoxicity. *Proc Natl Acad Sci U S A* **95:** 14500–14505.

Meucci O, Miller RJ (1996). gp120-induced neurotoxicity in hippocampal pyramidal neuron cultures: protective action of TGF-beta1. *J Neurosci* **16:** 4080–4088.

Neale JH, Olszewski RT, Gehl LM, Wroblewska B, Bzdega T (2005). The neurotransmitter N-acetylaspartylglutamate in models of pain, ALS, diabetic neuropathy, CNS injury and schizophrenia. *Trends Pharmacol Sci* **26:** 477–84.

Pin JP, Duvoisin R (1995). The metabotropic glutamate receptors: structure and functions. *Neuropharmacology* **34:** 1–26.

Robinson MB, Blakely RD, Couto R, Coyle JT (1987). Hydrolysis of the brain dipeptide N-acetyl-L-aspartyl-L-glutamate. Identification and characterization of a novel N-acetylated alpha-linked acidic dipeptidase activity from rat brain. *J Biol Chem* **262:** 14498–14506.

Sacktor N, Lyles RH, Skolasky R, Kleeberger C, Selnes OA, Miller EN, Becker JT, Cohen B, McArthur JC (2001). HIV-associated neurologic disease incidence changes: Multicenter AIDS Cohort Study, 1990–1998. *Neurology* **56:** 257–260.

Sanchez-Prieto J, Budd DC, Herrero I, Vazquez E, Nicholls DG (1996). Presynaptic receptors and the control of glutamate exocytosis. *Trends Neurosci* **19:** 235–239.

Sindou P, Couratier P, Esclaire F, Yardin C, Bousseau A, Hugon J (1994). Prevention of HIV coat protein (gp120) toxicity in cortical cell cultures by riluzole. *J Neurol Sci* **126:** 133-137.

Slusher BS, Vornov JJ, Thomas AG, Hurn PD, Harukuni I, Bhardwaj A, Traystman RJ, Robinson MB, Britton P, Lu XC, Tortella FC, Wozniak KM,

Yudkoff M, Potter BM, Jackson PF (1999). Selective inhibition of NAALADase, which converts NAAG to glutamate, reduces ischemic brain injury. *Nat Med* **5:** 1396–1402.

Stauch BL, Robinson MB, Forloni G, Tsai G, Coyle JT (1989). The effects of N-acetylated alpha-linked acidic dipeptidase (NAALADase) inhibitors on [3H]NAAG catabolism in vivo. *Neurosci Lett* **100:** 295–300.

Thomas AG, Liu W, Olkowski JL, Tang Z, Lin Q, Lu XC, Slusher BS (2001). Neuroprotection mediated by glutamate carboxypeptidase II (NAALADase) inhibition requires TGF-beta. *Eur J Pharmacol* **430:** 33–40.

Thomas AG, Vornov JJ, Olkowski JL, Merion AT, Slusher BS (2000). *N*-Acetylated alpha-linked acidic dipeptidase converts N-acetylaspartylglutamate from a neuroprotectant to a neurotoxin. *J Pharmacol Exp Ther* **295:** 16–22.

Tortella FC, Lin Y, Ved H, Slusher BS, Dave JR (2000). Neuroprotection produced by the NAALADase inhibitor 2-PMPA in rat cerebellar neurons. *Eur J Pharmacol* **402:** 31–37.

Vesce S, Bezzi P, Rossi D, Meldolesi J, Volterra A (1997). HIV-1 gp120 glycoprotein affects the astrocyte control of extracellular glutamate by both inhibiting the uptake and stimulating the release of the amino acid. *FEBS Lett* **411:** 107–109.

Winder DG, Conn PJ (1993). Activation of metabotropic glutamate receptors increases cAMP accumulation in hippocampus by potentiating responses to endogenous adenosine. *J Neurosci* **13:** 38–44.

Wroblewska B, Wroblewski JT, Pshenichkin S, Surin A, Sullivan SE, Neale JH (1997). *N*-acetylaspartylglutamate selectively activates mGluR3 receptors in transfected cells. *J Neurochem* **69:** 174–18.

This paper was first published online on Early Online on 07 December 2009.