Synergistic Neurotoxicity by Human Immunodeficiency Virus Proteins Tat and gp120: Protection by Memantine

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Human immunodeficiency virus type 1 (HIV-1) proteins Tat and gp120 have been implicated in the pathogenesis of dementia associated with HIV infection. Recently, we showed the presence of Tat protein in brains of patients with HIV-1 encephalitis as well as macaques with encephalitis caused by a chimeric strain of HIV and simian immunodeficiency virus, and that even transient exposure of cells to Tat leads to release of cytopathic cytokines. Now, we report the first demonstration of gp120 protein in brain of patients with HIV encephalitis. We tested the hypothesis that Tat and gp120 would act synergistically to potentiate each protein's neurotoxic effects and determined the extent to which pharmacological antagonists against processes implicated in HIV-1 neuropathogenesis could block HIV-1 protein-induced neurotoxicity. Subtoxic concentrations of Tat and gp120, when incubated together, caused neuronal cell death and prolonged increases in levels of intracellular calcium. A transient exposure of neurons to Tat and gp120 for seconds initiated neuronal cell death, but maximal levels of neuronal cell death were observed with exposures lasting 30 minutes. The neurotoxicity caused by Tat and gp120 applied in combination was blocked completely by memantine, partially by amiloride, and not at all by dipyridamole or vigabatrin.

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Infection with the human immunodeficiency virus (HIV) causes neuronal degeneration and frequently results in a dementing illness. The neuronal degeneration occurs although neurons themselves are only rarely infected, and this suggests that indirect mechanisms participate in neuronal demise. Cells predominantly infected with HIV-1 are invading macrophages and microglia, which release neurotoxic substances including the HIV-1 proteins Tat and gp120.[1] Tat, a nonstructural viral protein essential for viral replication, is actively released from unruptured cells,[2] is present in serum and brain of HIV-infected patients[3–5] and in macaques with encephalitis caused by a chimeric strain of HIV and simian immunodeficiency virus.[6] In vitro and in vivo studies have shown that Tat is neurotoxic (for review, see study by Nath and Geiger[1]). gp120, the HIV-1 envelope glycoprotein, has been detected in the serum and cerebrospinal fluid of HIV-1–infected patients[7,8] and is neurotoxic both in vitro and in vivo. However, despite findings that *env* mRNA is elevated in brain of patients with HIV-1 encephalitis[9,10] the role of gp120 in mediating HIV dementia has been questioned because of the inability to detect gp120 protein in brain.[11] By using highly specific polyclonal antisera, we now demonstrate the presence of gp120 in brain of patients with HIV-1 encephalitis and, in so doing, further establish the role of gp120 in the neuropathogenesis of HIV-1 infection.

Several mechanisms have been implicated in viral protein-induced neurotoxicity including oxidative pathways, excitotoxicity, sodium-proton exchange, calcium dysregulation, release of cytokines, and blockade of glutamate uptake.[1] Based on these observations, several drug studies have been initiated for the treatment of HIV dementia. In this study, we first tested the hypothesis that subthreshold doses of gp120 and Tat, when present together, would be sufficient and necessary to cause potentiated increases in levels of intracellular calcium and neurotoxicity. We next tested the hypothesis that the synergistic and neurotoxic actions of gp120 coadministered with Tat could be blocked by clinically available pharmacological antagonists and thereby not only potentially identify agents with therapeutic potential but also processes implicated in HIV-1 neuropathogenesis.

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Materials and Methods

Immunohistochemistry

Paraffin-embedded formalin-fixed sections from the temporal lobe, basal ganglia, and hippocampus of 3 patients with HIV encephalitis were immunostained with polyclonal goat anti-gp120 sera generated against recombinant gp120 from HIV-$_{SF2}$ that was provided as a gift by Chiron Corporation (Emoryville, CA). This antiserum recognized a single band at 120 kd by western blot analysis of HIV-infected cell lysates. For gp120 staining, sections were deparaffinized and hydrated in serial dilutions of ethanol. Sections were heated in a household microwave in 0.1 M sodium citrate (pH 6.0) for 10 minutes. Endogenous peroxidase was quenched by using 3% H_2O_2. Slides were incubated with the primary antisera (60 μg/ml) for 15 hours at 4°C. Biotinylated anti-goat IgG (1:500; Chemicon, Temecula, CA) was used as a secondary antibody followed by streptavidin horseradish peroxidase. Immunostaining intensity was amplified by using the tyramine signal amplification system per the manufacturer's protocol (NEN Life Sciences, Boston, MA). Diaminobenzidine was used as the chromogen. Brain tissues from two patients not infected with HIV-1 and without any known neurological complications, and another 3 patients with multiple sclerosis and active plaques were used as controls.

Neuronal Cultures

Brain specimens were obtained from human fetuses of 12 to 14 weeks' gestational age with consent from women undergoing elective termination of pregnancy and approval by the University of Kentucky Institutional Review Board and the University of Manitoba's Committee for Protection of Human Subjects. Neuronal cultures were prepared as described previously.[12,13] In brief, the cells were mechanically dissociated, suspended in Opti-MEM with 1% heat-inactivated fetal bovine serum, 0.2% N2 supplement (GIBCO) and 1% antibiotic solution (penicillin G 10^4 U/ml, streptomycin 10 mg/ml, and amphotericin B 25 μg/ml) and plated in flat-bottom 96-well plates. The cells were maintained in culture for at least 6 weeks before conducting the neurotoxicity and calcium imaging experiments.

Recombinant Tat and gp120 Proteins

Recombinant Tat was prepared as described previously[14] with minor modifications. The *tat* gene encoding the first 72 amino acids were amplified from HIV$_{BRU}$ obtained from Dr Richard Gaynor through the AIDS repository at the National Institutes of Health and inserted into an *Escherichia coli* vector PinPoint Xa2 (Promega). This construct allowed the expression of Tat as a fusion protein naturally biotinylated at the N-terminus. The biotinylated Tat protein was purified on a column of soft release avidin resin and cleaved from the fusion protein using factor Xa and eluded from the column followed by desalting with a PD10 column. All purification steps contained dithiothreitol to prevent oxidation of the proteins. Tat proteins were more than 95% pure, as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoreses followed by silver staining. Analysis by high-performance liquid chromatography, using a C4 column showed a single symmetrical peak. Western blot analysis showed that these preparations contained both monomeric and dimeric forms of Tat. The functional activity of Tat was confirmed a using a transactivation assay in HL3T1 cells containing an HIV-1 LTR-CAT construct.[14] gp120 from HIV$_{SF2}$ was obtained as a gift from Chiron Corporation. Recombinant gp120 was made in a Chinese hamster ovary cell line and purification yielded a product that was 95% gp120, with the remainder being breakdown products of gp120 as determined by western blot analysis. The Tat and gp120 preparations contained less than 1 pg/ml endotoxin, as determined by using a Pyrochrome Chromogenic test kit (Associates of Cape Cod, Falmouth, MA). The Tat protein was stored in a lyophilized form and gp120 as a stock solution in water at −80°C in endotoxin-free siliconized microfuge tubes until taken for experimentation. Tat and gp120 were highly susceptible to degradation and loss of biological activity with each freeze/thaw cycle. Therefore, single aliquots were used for each experiment with the remaining solutions discarded.

Neurotoxicity Assay

At the time of experimental treatment, the culture media was replaced with Locke's buffer containing (in mM) 154 NaCl, 5.6 KCl, 2.3 $CaCl_2$, 1 $MgCl_2$, 3.6 $NaHCO_3$, 5 glucose, 5 HEPES (pH 7.2), and neurons were incubated with Tat, gp120, or both proteins simultaneously. To determine if a transient exposure of neurons to the viral proteins was necessary and sufficient to cause toxicity, the viral proteins were incubated with the neurons for 30 seconds, 5 mintes, 30 minutes, or 15 hours followed by a complete media exchange. To determine if the responses were specific for the viral proteins, gp120 (30 pM) + Tat (60 nM) were serially incubated with protein A beads conjugated to Tat antisera and gp120 antisera or to protein A beads conjugated to normal rabbit sera. The supernatants were removed and incubated with neurons as described above. Cell death was monitored by trypan blue exclusion 15 hours after the change to Locke's buffer and the addition of HIV-1 protein(s) as described previously.[12,13] To determine mechanisms underlying Tat and gp120 neurotoxicity, cells were preincubated for 30 minutes with either memantine (2 μM), 5-(*N*-methyl-*N*-isobutyl)amiloride (MIA) (10 μM), dipyridamole (10 μM), or vigabatrin (20 μM) for 30 minutes before addition of Tat (60 nM) plus gp120 (30 pM). MIA was obtained from Sigma Chemical (St Louis, MO) and all other drugs were obtained from Tocris Cookson (Baldwin, MO). Cell death was monitored 15 hours after the addition of the HIV-1 proteins. Neuronal cell counts were determined from five fields at predetermined coordinate locations. Each field was photographed, coded, and counted. At least 200 cells were counted in each field. Each experiment was conducted in triplicate wells and at least two independent experiments were conducted with each pharmacological agent. The means and standard errors of the mean were calculated and data were analyzed by analysis of variance with Tukey-Kramer post hoc comparisons.

Intracellular Calcium Determinations

Levels of intracellular calcium ($[Ca^{2+}]_i$) were determined in human fetal neurons as previously described.[15] Coverslips containing fura-2–loaded cells were placed in a LUCSD Lei-

den coverslip dish situated in a PDMI2 open perfusion microincubator (Medical Microsystems, Greenvale, NY) and cells were superfused at 2 ml/min with Krebs buffer prewarmed to 37°C. Cells were excited at 340 and 380 nm, and emission was recorded at 510 nm with a video-based Universal imaging system (EMPIX, Missassauga, Ontario, Canada). R_{max}/R_{min} ratios were converted to nM $[Ca^{2+}]_i$ as described.[15] All images were acquired by real time averaging of 16 frames of each wavelength that included a background reference subtraction from each of the acquired images. gp120, Tat, or a combination of the two HIV-1 proteins were loaded into glass micropipettes (1.0 mm, outside diameter; 0.78 mm inside diameter) pulled to a final outer tip diameter of less than 1.0 μm. Micropipettes were positioned approximately three cell bodies away from target cells and HIV-1 proteins were pressure-applied to cells (3 × 100 msec, 6 psi) using a Picospritzer (General Valve, Fairfield, NJ). Cells within five cell body widths of the micropipette were monitored for time periods up to 1 hour. Peak increases in $[Ca^{2+}]_i$ were determined by subtracting the maximum $[Ca^{2+}]_i$ levels achieved during a 5 minute period after Tat or gp120 applications from baseline $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ levels were averaged over the cell body and large processes. Calcium dysregulation was defined as an inability of cells to maintain or reestablish calcium homeostasis 15 minutes after gp120 or Tat application. For all calcium experiments, the amount of Tat and gp120 pressure-applied to neurons was calculated in moles.

Results

Detection of gp120 in the Brain of Patients with HIV Encephalitis

Before testing the hypothesis that Tat was capable of synergistically increasing the neurotoxicity of gp120, we determined, using a new antibody against gp120, the presence of gp120 in the brains of patients with HIV-1 encephalopathy. This was an important first step, because the previous inability to detect gp120 in brain tissue of HIV-1–infected patients or in mice that transgenically overexpressed gp120 suggested questions as to whether gp120 played an important role in HIV-1 neuropathogenesis. The lack of staining of control tissues with this antisera indicated clearly a lack of immunoreactivity against normal cellular proteins or nonspecific staining because of breakdown of the blood–brain barrier. Gp120-positive cells were present in all HIV-1–infected patients tested and in all brain regions examined. Cells staining for gp120 were most often observed in basal ganglia and perivascular cells (Fig 1). Multinucleated giant cells were easily identified and were immunopositive for gp120 (see Fig 1A–C). gp120-positive cells with microglial morphology were found in close proximity to neurons (see Fig 1A) and were scattered in focal areas within white matter and basal ganglia (see Fig 1C and D). In some brain areas, gp120 immunoreactivity was also observed in the perivascular matrix (see Fig 1D). Occasionally, gp120-positive cells were noted in the lumen or wall of blood vessels (see Fig 1E and F).

Neurotoxicity of Tat and gp120 Is Synergistic

Tat (Fig 2A) and gp120 (see Fig 2B) dose-dependently increased neuronal cell death; however, gp120 induced neurotoxicity at concentrations two or three orders of magnitude less than Tat. For Tat, statistically significant levels of neurotoxicity were observed at concentrations starting at 125 nM (see Fig 2A). For gp120, statistically significant levels of neurotoxicity were observed at concentrations starting at 500 pM (see Fig 2B). To determine whether the neurotoxic properties of Tat and gp120 could be synergistic, we used the subthreshold gp120 concentration of 30 pM and the subthreshold Tat concentrations of 15, 30, or 60 nM. As expected from our previous studies, gp120 at 30 pM or Tat at 60 nM did not significantly increase neuronal death (Fig 3A). Significant neuronal cell death was observed when neurons were exposed to 30 pM gp120 in the presence of 60 nM Tat. There was a gradual decline in neuronal cell death with 30 and 15 nM concentrations of Tat in the presence of 30 pM gp120. The synergistic responses were obliterated by immunoabsorption of the viral proteins with antisera to Tat and gp120 but not by normal rabbit sera (see Fig 3B). In all instances, less than 15% cell death was observed and the dead cells were randomly scattered throughout the culture dishes. This latter finding suggests that a select population of neurons is susceptible to viral protein-induced neurotoxicity.

Intracellular Calcium Changes Induced by Tat Were Synergistic with gp120 and Glutamate

Tat (n = 45) at a dose of 200 fmol and gp120 (n = 53) at a dose of 0.01 fmol did not produce any significant changes in $[Ca^{2+}]_i$ in neurons (Fig 4), When these same doses of Tat and gp120 were combined and applied to neurons, immediate, transient increases in $[Ca^{2+}]_i$ of 173 ± 34 nM (n = 39) were observed. In 33% of these neurons, these initial increases in calcium were followed by dysregulation of calcium homeostasis apparent as secondary/prolonged increases in $[Ca^{2+}]_i$.

Next, we determined whether the synergistic responses observed between Tat and gp120 were specific to these two proteins or could be mimicked by a general excitotoxic stimulus like glutamate. As a control, we repeatedly exposed neurons to glutamate (100 μM) and found a steady decrement in calcium responses (Fig 5A). Initial peak $[Ca^{2+}]_i$ responses were 451 ± 52 nM, followed by 291 ± 30, 225 ± 23, and 174 ± 15 nM. However, after a transient exposure of neurons to 2 pmol of Tat, glutamate (100 μM)–induced increases in $[Ca^{2+}]_i$ were significantly ($p < 0.01$) larger (671 ± 64, 548 ± 43, 638 ± 34, and 459 ± 84 nM) and did not decrease with repeated exposures. In 17 of the 52

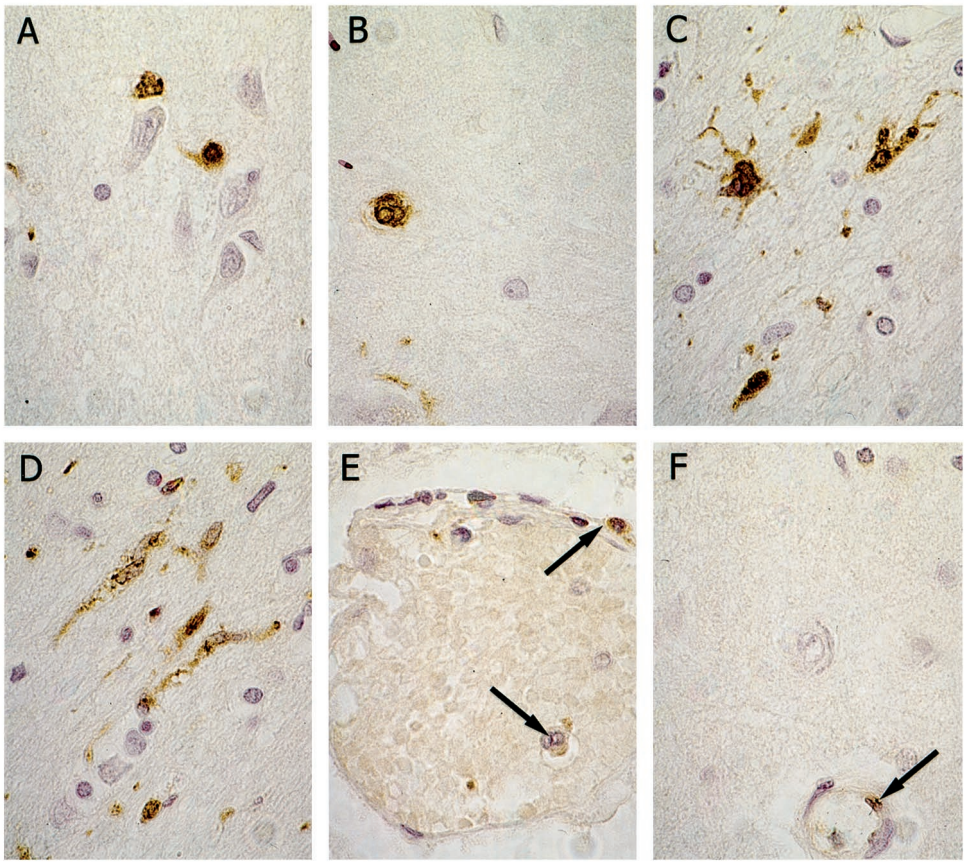

Fig 1. Immunohistochemical detection of gp120 in human immunodeficiency virus (HIV) encephalitis. Paraffin-embedded formalin-fixed sections from the basal ganglia, hippocampus, and temporal cortex of 4 patients with HIV encephalitis were immunostained with a polyclonal goat antisera raised against highly purified recombinant gp120. Diaminobenzidine was used as a chromogen. (A) gp120-positive cells in close proximity to neurons in the basal ganglia. (B) Multinucleated giant cell immunostained for gp120. (C) Several glial cells in the basal ganglia showed immunostaining for gp120. (D) Several perivascular cells and the perivascular matrix showed immunostaining for gp120. (E) A capillary in the basal ganglia showed gp120-positive cells in the lumen and in the perivascular region. (F) A small blood vessel in the hippocampal region showed gp120-positive cells.

cells tested, we observed large and dysregulated increases in $[Ca^{2+}]_i$ (see Fig 5B).

Neurotoxicity to Tat and gp120 Requires Only a Transient Exposure

To determine the length of time neurons must be exposed to viral proteins for inducing neurotoxicity, we incubated human fetal neurons with a combination of Tat and gp120 for 30 seconds, 5 minutes, 30 minutes, or 15 hours, and neuronal cell death was monitored 15 hours later after the proteins were applied to the neurons. Increased neuronal cell death was observed with 30 seconds and longer exposures to Tat and gp120. A 30-minute exposure was sufficient to produce nearly maximal levels of neuronal cell death (Fig 6).

Pharmacological Characteristics of Tat and gp120 Toxicity

Excitatory amino acid receptors, sodium-proton exchangers, free radicals, and γ-aminobutyric acid (GABA) have all been implicated in pathogenesis and treatment of HIV-1 dementia. Accordingly, we tested the ability of pharmacological inhibitors of these implicated systems to block the neurotoxicity induced by Tat in combination with gp120. The glutamate receptor antagonist memantine completely blocked HIV protein–induced neurotoxicity (Fig 7). The sodium-proton exchange blocker MIA partially blocked the neurotoxicity. However, neither the free radical scavenger dipyridamole nor the GABA agonist vigabatrin significantly decreased the HIV-1 protein–induced neurotoxicity.

Discussion

Tat, gp120, and other HIV-1 proteins can cause neurotoxicity in vitro as well as in vivo. However, demonstration of the presence of these proteins in the brains of HIV-1–infected patients has been difficult and it remains unclear as to whether sufficient quantities of these proteins are present to produce neurotoxicity in vivo. Some progress in this regard has been made in-

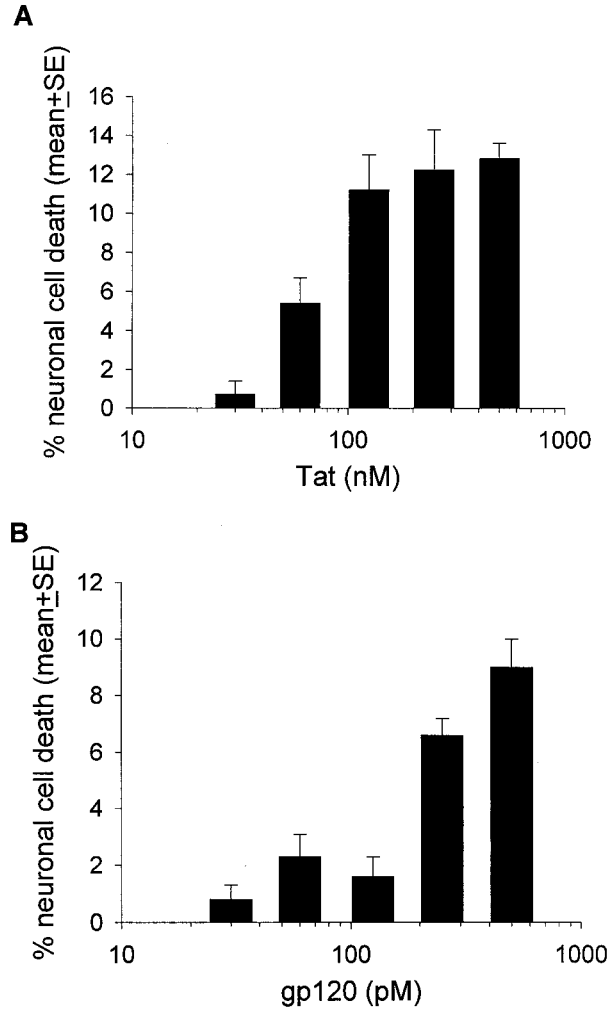

Fig 2. Dose–response of Tat- and gp120-induced neurotoxicity. Cultures of human fetal neurons were treated with either Tat (30–500 nM) or gp120 (30–500 pM) and cell death was monitored as described in Materials and Methods. All data represent percentages of neuronal cell death above control, calculated as mean ± SE values from three independent experiments performed in triplicate. Significant cell death ($p < 0.05$) was noted with ≥125 nM Tat (A) and 500 pM gp120 (B).

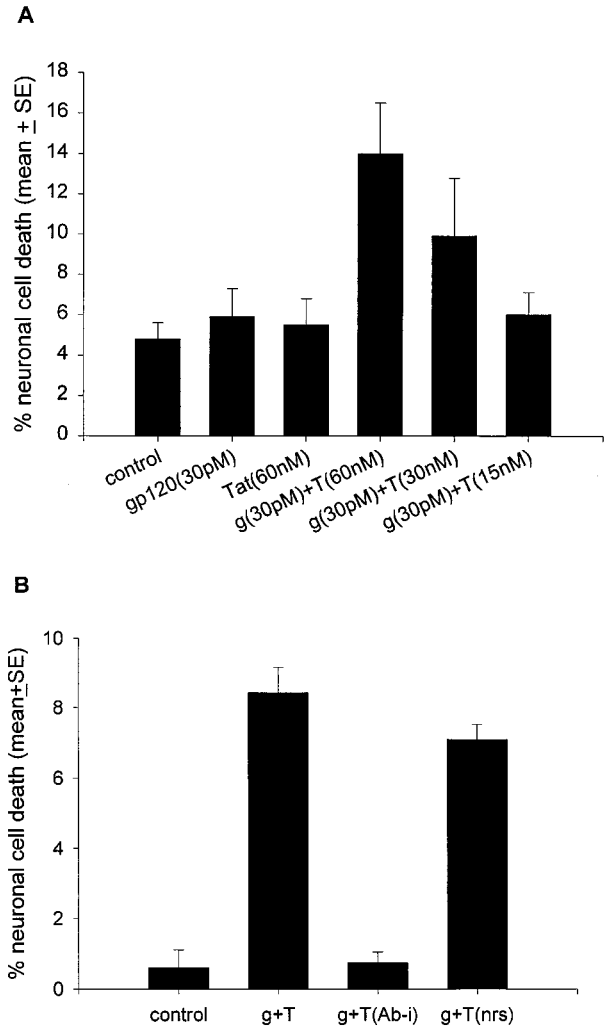

Fig 3. Synergistic response of Tat- and gp120-induced neurotoxicity. (A) Significant cell death ($p < 0.05$) was noted when human fetal neuronal cultures were treated with gp120 (30 pM) and Tat (60 nM) in combination, whereas neither dose produced significant toxicity when added independently. Progressively less neuronal cell death was noted when gp120 (30 pM) was added with either 30 nM or 15 nM concentration of Tat. (B) Neurotoxicity of gp120 (30 pM) + Tat (60 nM) was blocked by immunoabsorption with gp120 and Tat antisera but not by normal rabbit sera.

cluding reports by several independent laboratories that the HIV-1 Tat protein was present in brain of patients with HIV-1 encephalitis.[4,5] We now demonstrate immunohistochemically the presence of gp120 in microglial cells, multinucleated giant cells, and mononuclear perivascular cells in the brains of patients with HIV-1 encephalitis. This breakthrough was likely aided by the availability of a polyclonal antisera directed against the glycosylated form of gp120 and the use of a technique to amplify immunohistochemical staining. These observations provide an important missing link in establishing the role of these proteins in mediating HIV-1–associated neuronal dysfunction. We further demonstrate that only very small concentrations of HIV-1 proteins are required to induce neurotoxicity and that continuous exposure of neurons to these proteins is not needed to produce neurotoxicity.

Previous studies from our laboratory have shown that Tat and gp120 use different mechanisms for causing at least initial increases in levels of intracellular calcium in neurons, although in both instances these increases in intracellular calcium lead to cell death. Tat increased the release of calcium from inositol 1,4,5-trisphosphate–regulated intracellular pools and, subse-

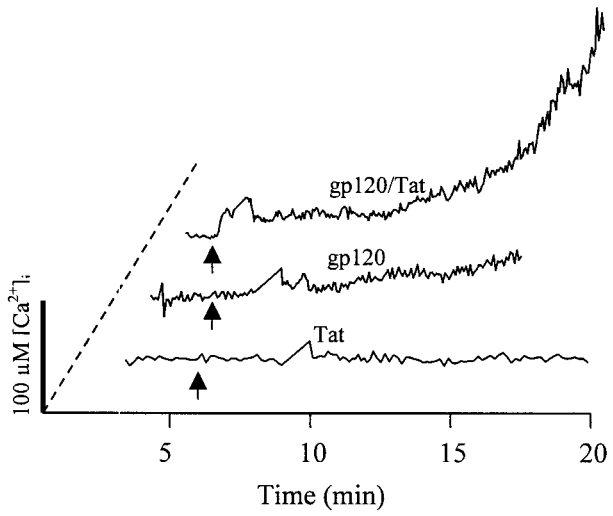

Fig 4. Synergistic effects of Tat and gp120 on $[Ca^{2+}]_i$ in cultured human fetal neurons. Tat (200 fmol) and gp120 (0.01 fmol) by themselves or in combination were loaded into micropipettes and pulse pressure applied onto neurons. Tat or gp120, when applied alone, produced small increases in $[Ca^{2+}]_i$. In contrast, when gp120 and Tat were applied together, significantly larger transient and secondary/prolonged increases in $[Ca^{2+}]_i$ were observed. The calcium transients illustrated were representative of 45 neurons tested with Tat, 53 neurons tested with gp120, and 39 neurons tested with Tat and gp120 in combination.

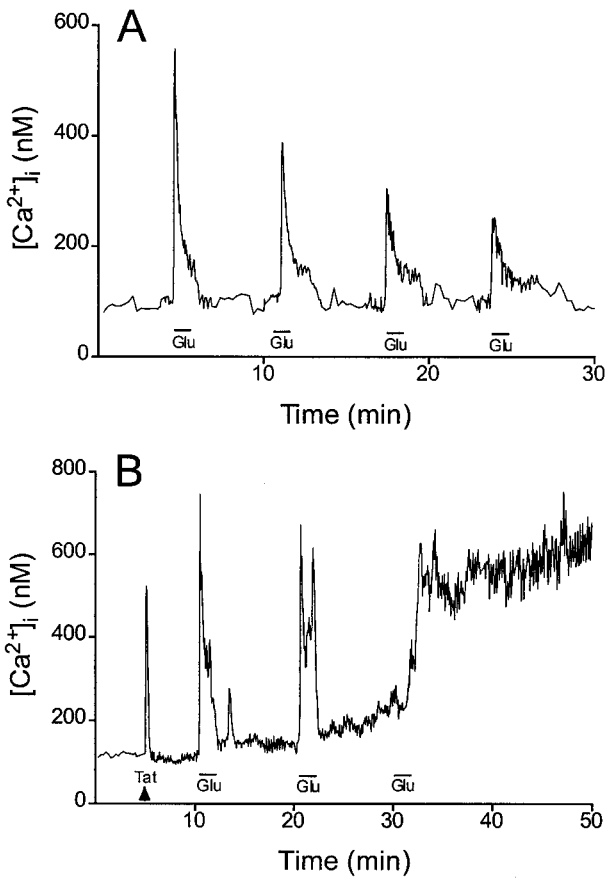

Fig 5. Increased sensitivity to glutamate by transient exposure to Tat. (A) Repeated applications of glutamate (Glu; 100 μM), interspersed by 10-minute wash periods, resulted in progressively smaller increases in levels of intracellular calcium. (B) Pressure application of 50 fmol Tat increased the sensitivity and resulted in massive dysregulation of calcium responses to glutamate. These illustrations are representative of 39 neurons tested with glutamate alone and 52 neurons preexposed to Tat followed by glutamate.

quent to this, the influx of calcium mediated by excitatory amino acid receptors. On the other hand, gp120 acted first on sodium-proton exchange channels and, subsequent to that action, calcium fluxed into cells mediated by L-type calcium channels and excitatory amino acid receptors.[15,16] Although the initial actions of Tat and gp120 were different, the convergence onto similar mechanisms for increasing levels of intracellular calcium led us to hypothesize that their combined actions might be more than additive (ie, synergistic). In this study, we observed that Tat and gp120, when incubated together at subtoxic and subthreshold doses, produced significant dysregulation of calcium and neuronal cell death. These synergistic responses were of such magnitude that toxic concentrations of Tat and gp120 in combination were about 10- to 20-fold lower than were concentrations of gp120 necessary to decrease neuronal viability.

Previously, it was reported that the neurotoxic effects of gp120 and glutamate were synergistic.[17] Now, we demonstrate that in the presence of Tat, glutamate produced massive calcium dysregulation in neurons. Thus, viral proteins can synergize with one another and with other neurotoxic substances to cause significant derangement of neuronal function at much lower concentrations than previously envisioned.

We demonstrated that exposure to viral proteins for seconds to minutes was sufficient to cause neurotoxicity several hours later. This delayed response after a transient exposure to Tat is consistent with previous observations. For example, exposure of glial cells and monocytes to Tat for a few minutes was sufficient to induce the expression of cytokines implicated in neurotoxicity several hours later.[18] Also, Tat injection intracerebroventricularly in rats produced progressive pathological changes although Tat could not be detected in brain after 2 hours.[19] Thus, continuous exposure to viral proteins may not be necessary and a "hit and run" phenomenon may be operative.

Our initial studies showed that Tat-induced neurotoxicity occurred in the 0.5 to 1 μM range.[12] That study used Tat from a commercial source. We have since determined that several technical factors accounted for these high concentrations; Tat activity is

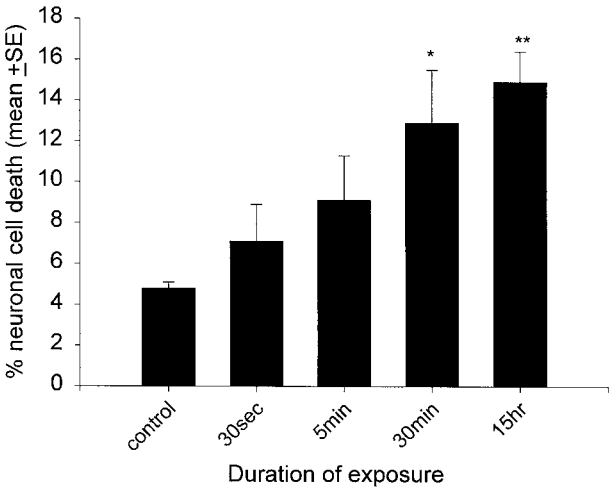

Fig 6. Neurotoxicity induced by transient exposure to Tat and gp120. Neuronal cell death was assessed at 15 hours after exposure to Tat (60 nM) and gp120 (30 pM) for variable durations of time. The amount of neuronal cell death correlated with the duration of Tat plus gp120 exposure. A 30-minute exposure was sufficient to cause significant neurotoxicity. $^{*}p < 0.05$; $^{**}p < 0.005$.

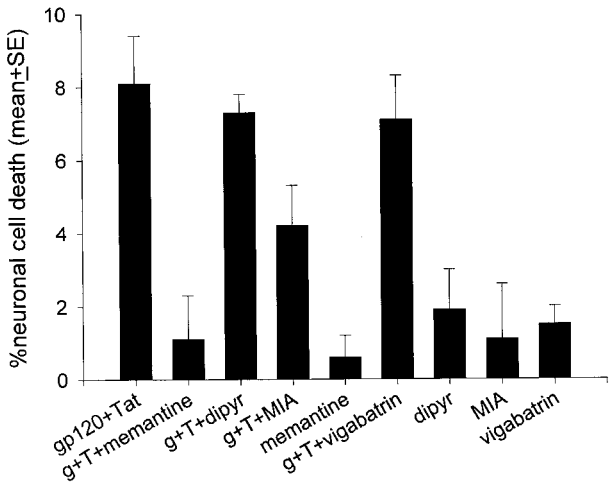

Fig 7. Pharmacological manipulation of Tat- and gp120-induced neurotoxicity. Neurotoxicity of Tat (T; 60 nM) plus gp120 (g; 30 pM) was completely inhibited by memantine (2 μM) ($p < 0.01$)*, partially inhibited* ($p < 0.05$) *by methylisobutylamiloride (MIA; 10 μM), and was not significantly affected by dipyridamole (dipyr; 10 μM) or vigabatrin (20 μM).*

highly susceptible to freeze/thaw cycles, it is easily oxidized, and it sticks to serum proteins, glass, and plastic with high affinity. Some of these factors are now controlled for and procedural changes have resulted in lower concentrations of Tat required to produce neurotoxicity. Factors that we are still unable to control are sticking of Tat to plastic dishes, metal needles of syringes, and some degradation of the molecule during purification and storage. We thus conclude that the actual in vivo concentrations for Tat-induced effects in the brain are probably much less than that needed for the in vitro experiments. Despite this, we were able to demonstrate effects of Tat on cytokine and chemokine induction with concentrations of 10 to 100 ng/ml of Tat,[18] which are similar to the concentrations found by others to alter physiological effects.[4,20–31] Furthermore, the concentrations used by others and us are not too dissimilar from the concentrations of 1 ng/ml of Tat that were shown to be present in the serum of patients with HIV-1 infection[3] and 4 ng/ml in conditioned medium of HIV-1–infected cells.[32] Even so, levels of Tat directly adjacent to Tat-producing cells will be much higher than what is measured in biological fluids. Therefore, the levels of Tat necessary to cause neurotoxicity in vitro are close to levels found in vivo but likely are underestimates of levels in close proximity to HIV-1–infected/Tat-producing cells.

We used Tat and gp120 derived from lymphotropic strains of HIV. The neurotoxic domain of Tat is well conserved across all strains of HIV.[13,33] In a similar manner, no major differences were identified in the neurotoxic properties of gp120 from lymphotropic and monocytotropic strains of HIV.[34] Some differences in potency have been attributed to the cellular uptake properties of Tat governed by the second exon[14] and to the V3 loop of gp120.[35]

In this study, using cortical neuronal cultures, we noted that select populations of cells were susceptible to viral protein–induced neurotoxicity. These observations support previous in vivo studies in patients with HIV encephalitis, which showed that large cortical neurons, interneurons in the hippocampus, and nigrostriatal fibers were preferentially lost.[36–38] Further studies are necessary to determine the biochemical characteristics of these neurons.

We evaluated the neuroprotective role of several pharmacological agents with diverse mechanisms of action to determine underlying mechanisms for Tat and gp120 neurotoxicity. Memantine, a wide-spectrum glutamate antagonist completely blocked neurotoxicity produced by Tat and gp120 applied in combination. A previous study showed that memantine inhibited gp120-induced neurotoxicity.[39] Memantine is currently in clinical trial for the treatment of HIV-1 dementia. The effects of gp120 on intracellular calcium have been shown to be mediated via sodium-proton exchange channels, which can be blocked by amiloride.[40] Hence, we examined the ability of MIA, a potent analog of amiloride, to block Tat- and gp120-induced neurotoxicity. This drug only partially blocked the neurotoxic effects of the HIV proteins. To determine if inhibition of free radical production was an effective strategy for blocking Tat- plus gp120-induced

neurotoxicity, we used dipyridamole, which is available as an antiplatelet agent but also has antioxidant properties. We previously reported that dipyridamole blocked gp120-induced free radical production in monocytes.[41] Tat also causes an increase in free radical production by causing mitochondrial dysfunction in neurons and inhibiting manganese superoxide dismutase.[42,43] However, dipyramidole had no effect on Tat- plus gp120-induced neurotoxicity. This does not, however, exclude the possibility that other antioxidants may have a role in neuroprotection against the HIV proteins. Finally, we evaluated the role of a GABA agonist, vigabatrin, to block Tat- plus gp120-induced neurotoxicity, because patients with HIV encephalitis exhibit loss of GABA-containing interneurons in the hippocampus[36] and we have shown previously a relative increase in GABA levels in animals injected with Tat, presumably as a compensatory mechanism for the neurotoxic effects of the protein.[19] No neuroprotective effect of vigabatrin was noted.

In conclusion, both Tat and gp120 are present in the brains of patients with HIV encephalitis; their neurotoxic effects are synergistic and require only a transient expression. Further, the neurotoxicity induced by these products can be effectively blocked by memantine. Our studies support the exploration of this and similar drugs in the treatment of HIV dementia.

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