Neonatal Intrahippocampal Glycoprotein 120 Injection: The Role of Dopaminergic Alterations in Prepulse Inhibition in Adult Rats

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ABSTRACT

Following neonatal hippocampal administration on postnatal day 1, the dose-response effects of the human immunodeficiency virus 1 protein glycoprotein 120 (gp120) were studied in vivo on prepulse inhibition (PPI) in adulthood. Furthermore, the role of dopaminergic alterations was examined as a within-subject factor. Using a randomized-block design, male and female pups of eight Sprague-Dawley litters were injected bilaterally with either vehicle (1 μl volume) or gp120 (1.29, 12.9, or 129 ng/μl). At 9 months of age, rats were injected s.c. with saline (SAL) (0.1 ml/kg) and tested on preattentive processes, as indexed by sensorimotor gating. Sensorimotor gating was measured by PPI of the auditory startle response (ASR) [interstimulus intervals (ISIs) of 0, 8, 40, 80, 120, and 4000 ms, six trial blocks, Latin square design]. One month later, the animals were treated with a D1/D2 agonist, apomorphine (APO) (0.1 mg/kg) and again tested for PPI. A significant attenuation of the baseline ASR by APO was noted. No significant effects were noted on control ASR trials (ISIs, 0 and 4000 ms). For the SAL condition, response inhibition was significantly reduced as a function of gp120 dose, and the inflection of the inhibition curve was significantly altered for the high-gp120 dose-treated animals. A gp120 treatment × APO drug interaction was evident on amplitude, but not latency, of the response inhibition, with an enhanced inhibition in the APO condition, collapsed across ISIs (08–120 ms) as the neonatal-injected gp120 dose increased. Use of APO to probe integrity of the dopaminergic system suggests long-lasting alterations in neuronal responses consequent to neonatal gp120 exposure.

Infection by human immunodeficiency virus (HIV) type 1 is often complicated by a variety of neuropsychological deficits, indicating minor cognitive motor disorders and HIV-1-associated dementia complex (HAD) (Bussiere et al., 1999). Cognitive impairments that are linked with HAD include poor attentional abilities, deficits in memory, and reduced alertness (Nath et al., 2000a). Research indicates a close link between HAD and sensory gating, indexed by event-related brain potentials, with event-related brain potential being a behavioral measure of central nervous system (CNS) activity and is measured by the reduction in startle reflex amplitude that occurs when the startling stimulus follows a weak prepulse, i.e., prepulse inhibition (PPI) (Hoffman and Ison, 1980).

One unique feature of HIV-1-induced neurodegeneration is that neurons are not productively infected by HIV-1, suggesting the mechanism of HIV-1-induced neuronal cell loss occurs indirectly. Numerous studies suggest that the development of HAD is mediated by regulatory (tat, rev) and structural (gp120, gp41) viral gene products of the virus that interact with neurons in the CNS (Bansal et al., 2000; Nath et al., 2000b). The gene products are released from HIV-1-infected cells, such as macrophages/microglia, and are then present extracellularly in the HIV-1-infected brain. The envelope glycoprotein 120 (gp120) and the transactivator of transcription (Tat) are likely agents of the observed neuronal loss in the brains of AIDS patients and have been measured...
in the brain tissue of patients with HAD (Jones et al., 2000; Valle et al., 2000).

Support for the detrimental effects of gp120 on neurons has arisen particularly from animal and tissue culture studies (Brenneman et al., 1988; Lipton et al., 1991, 1995; Barks et al., 1997). Gp120 has been reported to produce death in rodent hippocampal neurons, retinal ganglion cells, and cerebellar granule cells, with the induced neurotoxicity being primarily mediated by N-methyl-D-aspartate (NMDA) receptor mechanisms (Brenneman et al., 1988; Lipton et al., 1991). By binding to and/or indirectly activating cell surface receptors such as CXCR4 and NMDA, the HIV-1 protein gp120 may trigger neuronal apoptosis and excitotoxicity as a result of oxidative stress, perturbed cellular calcium homeostasis, and mitochondrial alterations. Thus, gp120 is one of the HIV-1 proteins that plays a significant role in the pathogenesis of neuronal damage and development of neuropsychological impairment.

Recent findings give evidence for selective sensitivity of dopamine (DA) neurons to HIV-1 protein neurotoxins and possible modulation of Tat and gp120 toxicity by drugs that act on the DAergic system (Nath et al., 2000a; Aksenov et al., 2006; Aksenova et al., 2006). It is suggested that DA alterations may amplify the neurological manifestation of HIV-1 infection. Damage to the DAergic system plays a significant role in the development of dementia in the brain of HIV-1 patients but also in Parkinsonian patients (Berger and Arendt, 2000). Symptoms of HAD, such as apathy, bradykinesia, psychomotor slowing, and an altered posture and gait, are similar to those observed in advanced Parkinson’s disease and suggest a profound abnormality of the striatal DAergic systems.

Sensorimotor gating, as indexed by PPI, has revealed significant alterations following intrahippocampal injections of Tat in adult or neonatal rats (Fitting et al., 2006a,b). In contrast, findings for gp120 have not been as compelling. Administration of gp120 to neonatal rats failed to alter developmental milestones, including measures of physical and sensory development as well as behavior mediated by simple reflex circuits (Hill et al., 1993). The finding of limited effects of neonatal gp120 exposure on the sensorimotor system is supported by a study specifically examining sensorimotor gating, as indexed by PPI in weanling rats (S. Fitting, R. M. Booze, and C. F. Mactutus, unpublished data). Likewise, gp120 exposure in utero and/or s.c. injection of gp120 during the neonatal period failed to cause measurable neurotoxicity or developmental toxicity (Bussiere et al., 1999). No effects were reported on survival, body weight, food consumption, reflex and physical development, behavioral performance (functional observational battery, motor activity, auditory startle habituation, passive avoidance, escape in a water M-maze), or on neuropathology. These findings raise the question about the role of gp120 in vivo and its contribution to the neuropsychiatric deficits seen in HIV-1-infected patients with HAD, including cognitive dysfunctions such as attentional deficits (Nath et al., 2000a). The role of the DAergic system in sensorimotor gating deficits is supported by numerous preclinical studies and is an accepted model in research investigating the underling mechanism in schizophrenic patients (Braff et al., 1978).

The purpose of this study was 3-fold: to determine long-lasting dose-dependent gp120 effects in PPI of adult rats; to uncover latent effects of gp120 by using a D_1/D_2 agonist, APO; and to use APO as a tool to examine the integrity of the DAergic system in neonatal gp120-treated rats.

Materials and Methods

Animals

Sprague-Dawley pregnant dams (n = 8) were obtained from Harlan Laboratories, Inc. (Indianapolis, IN) and delivered to the vivarium before embryonic day 7. Dams were single-housed with food (Pro-Lab Rat, Mouse Hamster Chow no. 3000, National Institutes of Health diet no. 31), and water was available ad libitum. Females were checked twice daily for the onset of parturition as they approached term. The day pups were found in the cage was designated as postnatal day (P) 0. On P1, litters were culled to 10 offspring with an equal sex ratio, if possible. No more than one female or one male per litter were assigned to a single condition. At 21 days of age, animals were weaned, separated by sex, and pair housed throughout the experiment. The animal facility was maintained at 21 ± 2°C, 50 ± 10% relative humidity, and had a 12-h light/dark cycle with lights on at 7:00 AM (East Standard Time). The animals were maintained according to the National Institutes of Health guidelines in Association for Assessment and Accreditation of Laboratory Animal Care-accredited facilities. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of South Carolina, Columbia.

Experimental Design

A randomized block design was employed, with litter as the blocking factor, in which all experimental gp120-dose treatments were represented. Thus, animals were randomly assigned to one of four gp120 treatment groups that received bilateral hippocampal injections at P1 of 1 μl of vehicle (0 ng of gp120, n = 5), 1.29 ng of gp120 as the low-dose group (n = 5), 12.9 ng of gp120 as the medium-dose group (n = 5), or 129 ng of gp120 as the high-dose group (n = 8). In each of the groups, male and female rats were included. At 9 months of age animals received s.c. SAL injections and were tested for sensorimotor gating by assessing PPI of the acoustic startle response (ASR). One month later, to test the integrity of the DAergic system, the same adult rats were s.c. injected with APO and were again tested for PPI. Testing occurred between 9:00 AM to 5:00 PM. Following completion of behavioral testing, animals were euthanized (pentobarbital overdose), and brains were removed from the skull. Cryostat-cut sections (20 μm) through the hippocampal injection sites were collected. Analysis of Nissl-stained sections confirmed and verified placement of injection sites into the hippocampal dentate hilar region.

Dose-Response gp120 Study

Purified recombinant HIV-1 gp120 LAV (IIIB), MN, CM envelope protein was purchased from Protein Sciences Corp. (Meriden, CT) with a concentration of 100 μg at 1.0 ml. Gp120 was stored at −20°C until used for testing. Solutions of gp120 were prepared to get the following three doses: 1.29, 12.9, or 129 ng/μl. These three doses of gp120 were below the threshold for gross behavioral deficits. Doses of 250 ng of gp120 or higher are necessary to produce significant loss of striatal tissue and significantly increase the number of glial fibrillary acidic protein-reactive cells (Bansal et al., 2000).

Surgery

Standard stereotoxic surgery techniques modified for neonates were used for gp120 treatment injections. Individual pups were removed from the dam and cryogenically anesthetized before being placed in a modified stereotoxic holder for surgery of neonates (Kopf, Inc., Tujunga, CA), which included a chilled base to maintain cryogenic anesthesia. Rubber head bars held the skull in place while bilateral microinjections of VEH (physiological saline) or gp120 were
made directly into the hippocampus using the stereotoxic coordinates for injections and microsyringes (Hamilton Co., Reno, NV; microliter no. 701 RN, 10 μl). The set of coordinates used for the hippocampus were: right hemisphere, −0.3 mm anterior to the bregma, 0.7 mm lateral to bregma, −3.0 mm dorsal from dura; and left hemisphere, −0.3 mm anterior to the bregma, −0.7 mm medial to bregma, −3.0 mm dorsal from dura. The VEH and gp120-treated animals were injected with the same volume (1 μl), and gp120 was dissolved in saline. The 1-μl injection volume was released over 1 min after a 1-min resting period that allowed the tissue to return to its original conformation. The injection needle was withdrawn over 2 min to prevent reflux. After the two injections, the piercings in the skin of the head were closed with surgical glue, and the pups were warmed under a heat lamp (35°C) before being returned to the dam, where they were closely observed for indications of rejection. No pups were rejected or abused by the dam. gp120 had no significant effect on body weight or growth.

ASR and PPI Assessment

Apparatus. The startle chamber (SR-Lab Startle Reflex System, San Diego Instruments, Inc., San Diego, CA) was enclosed in a 10-cm-thick double-walled, 81 × 81 × 116-cm isolation cabinet (external dimensions) (Industrial Acoustic Company, Inc., Bronx, NY). Each animal was tested individually in the dark with a high-frequency loudspeaker, that produced a background white noise (70 dB(A)) and was mounted inside the chamber 31 cm above the Plexiglas cylinder. The startle chamber consisted of a Plexiglas cylinder, 8.75 cm in internal diameter resting on a 12.5-× 20-cm Plexiglas stand. The stimulus was 100 dB(A) and the prepulse stimulus was 85 dB(A) in intensity. Both stimuli had a duration of 20 ms. The response of the animal to the startle stimulus produced deflection of the Plexiglas cylinder, which was converted into an analog signal by a piezoelectric accelerometer. Acoustic stimulus intensities were measured and calibrated with a sound level meter (Extech Instruments, Waltham, MA) with the microphone placed inside the Plexiglas cylinder. Response sensitivities were calibrated using a SR-LAB Startle Calibration System. The response signals were then digitized (12 bit A to D) and saved to a hard disk on a Pentium class computer.

Testing Procedures. All rats were tested for approximately 20 min. Animals were first exposed to a 5-min acclimation period of 70 dB(A) background of white noise, followed by six single white noise stimuli of 100 dB(A) and 36 PPI trials with 0-, 8-, 40-, 80-, 120-, and 4000-ms interstimulus intervals (ISIs), assigned by Latin square design. The stimulus duration was 20 ms. The six single stimuli assessed the baseline ASR without a prior prepulse stimulus and the PPI trials 0- and 4000-ms ISIs were defined as control trials to provide the baseline ASR within the PPI test. The ISI, therefore, represents the time from the offset of the prepulse stimulus to the onset of the startle stimulus. For PPI, the dependent measures analyzed were peak ASR amplitude, peak ASR latency (from startle stimulus onset to the peak response), and percent PPI. Percent PPI is commonly expressed as percentage of inhibition in startle amplitude at the 100-ms ISI (Geyer and Swerdlow, 2004) and was computed according to the following formula: percent PPI = [(0-μs ISI trials – (80 - 120-μs ISI trials))2/0-μs ISI trials] × 100.

Dose-Response Apomorphine Study

Apomorphine hydrochloride (APO) was purchased from Sigma (St. Louis, MO). A preliminary dose-response study was conducted to investigate the effects of APO on sensorimotor gating (S. Fitting and C. F. Mactutus, unpublished data). In this study, three doses were used, 0.1, 0.25, and 0.5 mg/kg APO. Data indicated a quadratic function with the most robust PPI-disruptive effects for the 0.1 mg/kg APO dose (data not shown). This finding is supported by other studies that have shown robust PPI-disruptive effects in Hayman Sprague-Dawley rats produced by a dose of 0.1 mg/kg APO (Swerdlow et al., 2000). In the present experiment, the dosing regimen of 0.1 mg/kg APO was employed. APO (0.1 mg/kg) was administered s.c. to rats 5 min prior to PPI testing. The dose of APO hydrochloride (0.1 mg/kg; Sigma) was calculated on the weight of the salt and dissolved in saline prior to injection in a volume of 1 ml/kg.

Data Analysis

All data were analyzed using analysis of variance (ANOVA) techniques (Winer, 1971). Two-way mixed-factor ANOVAs, with gp120 treatment as a between-subjects factor (0, 1.29, 12.9, or 129 ng of gp120) and APO drug as a within-subjects factor (SAL, APO), were performed on peak ASR amplitude and peak ASR latency in the baseline ASR trials, control trials, and on percent PPI (at 100-ms ISI). For PPI trials (8–120-ms ISIs) three-way mixed-factor ANOVAs were conducted with adding PPI trials as a within-subjects factor. For the within-subjects terms, potential violations of sphericity (Winer, 1971) were preferentially handled by the use of orthogonal decomposition or, if necessary, the use of the Greenhouse-Geisser df correction factor. Orthogonal component analyses of PPI trials separate for each gp120 treatment were employed to evaluate the nature of the PPI trial-dependent effects. An added positive use of the orthogonal component analyses is that this statistic also describes the shape of the function by determining its significance (e.g., linear, quadratic, etc. equations). A significant main effect or interaction will thus be described with its significant components. Planned comparisons, with comparing the protein gp120 treatments against the VEH group as a single control mean, were conducted to determine specific gp120 treatment effects. In addition, for peak ASR amplitude, the ISI at which the maximal inhibition response occurred was recorded across all PPI trials (8–120-ms ISIs). ISI data are categorical in nature; thus, the Fisher’s exact test was applied. An a level of p < 0.05 was considered significant for all statistical tests.

Results

ASR Test

For the baseline ASR a gp120 treatment × APO drug mixed-model ANOVA on peak ASR amplitude revealed a significant gp120 treatment effect [F(3, 20) = 6.3, p < 0.01] and a significant APO drug effect [F(1, 20) = 7.1, p < 0.02], but no gp120 treatment × APO drug interaction. For gp120 treatment, cubic and quadratic components were noted [F(1, 20) = 14.0, p < 0.01 and F(1, 20) = 5.1, p < 0.04, respectively]. Likewise, comparing each of the protein gp120 treatments against the VEH-treated animals, only a significant difference with the 12.9 ng of gp120 dose was revealed (p < 0.01). Furthermore, APO attenuated the baseline of peak ASR amplitude but did not significantly alter gp120 neurotoxicity (Fig. 1). A gp120 treatment × APO drug mixed-model ANOVA on peak ASR latency revealed no gp120 treatment or APO drug effect or interaction between gp120 treatment and APO drug.

PPI Test

Control Trials (0- and 4000-ms ISIs Combined). A gp120 treatment × APO drug mixed-model ANOVA on peak ASR amplitude and peak ASR latency revealed no significant effects (data not shown).

Percent PPI (100-ms ISI). A gp120 treatment × APO drug mixed-model ANOVA conducted on percent inhibition of the peak ASR amplitude for ISI 100-ms revealed no significant effects, suggesting that the inhibition response was not altered by APO drug and gp120 treatment at any of the given gp120 doses.

PPI trials (8–120-ms ISIs). Overall, a gp120 treatment ×
APO drug \times \text{PPI trials mixed-model ANOVA conducted on peak ASR amplitude revealed no overall gp120 treatment but a significant APO drug effect } [F(1, 20) = 6.9, \ p \leq 0.02]. \text{ The gp120 treatment } \times \text{ APO drug interaction was significant } [F(3, 20) = 3.6, \ p \leq 0.03] \text{ with a linear component } [F(1, 20) = 10.1, \ p \leq 0.01], \text{ indicating that gp120 treatment was significantly altered by APO drug (Fig. 2).}

For the SAL condition, separate orthogonal component analyses for APO drug revealed a significant linear effect \ [F(1, 20) = 5.4, \ p \leq 0.03], \text{ with an enhanced response as gp120 dose increased, indicating a reduced inhibition response. A leftward peak shift in the inhibition function for the high gp120-dose group compared with the VEH-treated animals across all PPI trials was further noted } [\chi^2(1) = 4.1, \ p \leq 0.04], \text{ suggesting an alteration in the inflection of the inhibition curve (Fig. 3).}

For the APO condition, in contrast, a prominent linear component was noted that decreased as gp120 dose increased \ [F(1, 20) = 6.2, \ p \leq 0.02], \text{ indicating an enhanced inhibition response (Fig. 2).}

For the PPI trials, the mixed-model ANOVA revealed a significant overall PPI trial effect with a prominent linear effect \ [F(1, 20) = 43.1, \ p \leq 0.001] \text{ that significantly interacted with APO drug } [F(3, 20) = 4.6, \ p \leq 0.04, \text{ linear}] \text{ but not with gp120 protein treatment. In addition, Fisher’s exact probability tests on the ISIs in which the peak occurred were conducted. VEH-treated animals revealed no APO drug effect (Fig. 4A), whereas for the high-gp120 dose-treated animals (129 ng of gp120), APO revealed a significant decrease in peak response and an alteration in the inflection of the inhibition curve with a rightward peak shift } [\chi^2(1) = 4.3, \ p \leq 0.04] \text{ (Fig. 4B). No effect was noted between VEH-treated}

Fig. 1. Mean (± S.E.M.) peak ASR amplitude for the baseline ASR test with a significant main effect of gp120 treatment \ (p \leq 0.01) \text{ and APO drug } (p \leq 0.02). \text{ Significant effects for the planned comparisons against the VEH group are indicated. **, } p \leq 0.01.

Fig. 2. Mean (± S.E.M.) peak ASR amplitude in PPI trials collapsed across ISIs (8–120 ms) as a function of gp120 treatment and APO drug. A significant gp120 treatment \times APO drug interaction with a prominent linear component was noted } (p \leq 0.01), \text{ with a reduced response inhibition in the SAL condition and an enhanced inhibition response in the APO condition as gp120 dose increased.}

Fig. 3. Mean (± S.E.M.) peak ASR amplitude in PPI trials across ISIs (0–4000 ms) for the SAL condition. A leftward peak shift in the inhibition function for the high gp120-dose group compared with the VEH-treated animals across all PPI trials (8–120-ms ISIs) was noted } (p \leq 0.04).

Fig. 4. Mean (± S.E.M.) peak ASR amplitude in PPI trials across ISIs (0–4000 ms). A, VEH-treated animals in SAL versus APO condition. B, high-gp120-dose group in SAL versus APO condition with a significant peak shift } (p \leq 0.04). C, VEH-treated animals in SAL versus high-gp120-dose group in APO.
animals in the SAL condition and APO high gp120-dose-treated animals (Fig. 4C), suggesting masking or perhaps a mitigation of gp120 neurotoxicity with restoration toward normal by APO.

A gp120 treatment × APO drug × PPI trials mixed-model ANOVA conducted on peak ASR latency revealed only a significant PPI trial effect with a prominent linear component \( [F(1, 20) = 96.5, p < 0.001] \), indicating a linear slowing in response with an increase in ISIs between the prepulse and the startle stimulus (data not shown). No other significant effect was noted, suggesting that gp120 and APO did not alter ASR latency response.

**Discussion**

The present experiment indicated potential alterations in sensorimotor gating in adult rats, as measured by PPI, following neonatal bilateral intrahippocampal administration of gp120. Behavioral testing revealed alterations on ASR baseline and PPI trials for peak ASR amplitude but not peak ASR latency. A masking or perhaps a mitigation of long-term gp120-induced effects with restoration toward normal were evident by altering the DAergic system using 0.1 mg/kg APO, suggesting an impact on the integrity of the DAergic system by neonatal HIV-1 gp120 protein exposure.

On the baseline ASR, indexed by 36 startle reflex trials, gp120 treatment and APO drug significantly altered the peak ASR amplitude. A potentiation of the startle reflex was noted by a 12.9-ng gp120 dose but not by a low- and high-gp120 dose (1.29 and 129 ng of gp120, respectively). The observed elevation of the startle amplitude in the baseline ASR for hippocampal-related lesions is supported by other studies assessing adult rats after ibotenic acid lesions of the ventral hippocampus (Swerdlow et al., 1995). However, the nonmonotonic gp120-induced neurotoxic function on the baseline ASR assessed in the present study is not well understood and needs to be further examined.

For APO the baseline ASR was attenuated compared with the SAL condition in all four gp120 treatment groups (0, 1.29, 12.9, and 129 ng of gp120). An attenuation of the startle response by APO suggests a decreased responsivity to stimulus input and how the organism processes stimulus events. The attenuated startle baseline in the VEH-treated group remains in contrast to studies that report no APO effects on baseline ASR in nontreated rats (Davis et al., 1990) or excitatory effects of APO on startle amplitude in stimulus-only trials (Davis et al., 1990; Swerdlow et al., 2005). Interestingly, the mentioned studies report much higher APO doses, such as 0.5 (Swerdlow et al., 2005) or 0.8 (Davis et al., 1990) mg/kg. It is important to note that dose-response studies of APO on ASR in Sprague-Dawley rats confirm that APO does not follow a linear dose-response function (0–0.5 mg/kg APO doses) but rather an “inverted-U” dose function (Martinez et al., 2000). Thus, the dose of APO appears to be a crucial factor in determining how the startle reflex is affected by APO.

Alterations produced by gp120 on PPI trials (ISIs, 8–120 ms) were most evident in the peak ASR amplitude with a significant gp120 treatment × APO drug interaction. In the SAL condition, a significant reduction of the inhibition response as a function of gp120-dose was noted. In addition, the inflection of the inhibition curve was significantly altered for the high gp120 dose-treated animals. These results are in agreement with findings that demonstrated HIV-1 protein Tat alterations in the ISI function across PPI trials in adult rats (Fitting et al., 2006a) and neonatal rats throughout development (Fitting et al., 2006b). It should be noted that the significant gp120-induced alterations in adult rats in the present study are, however, greater than that seen in gp120-treated neonatal rats with the same doses but tested at weanlings (S. Fitting, R. M. Booze and C. F. Mactutus, unpublished data). The alterations observed in adulthood, but not at the age of weaning, suggest long-term effects of gp120. Neurotoxicity induced in vivo by gp120 has been reported to be mediated primarily by NMDA-type excitatory amino acid receptor mechanisms that interact with voltage-dependent Ca\(^{2+}\) channels (Barks et al., 1997). Excessive activation of NMDA receptors results in increased calcium concentrations followed by nitric oxide generation, which may finally induce neuronal apoptosis (Haughey and Mattson, 2002). Corasaniti et al. (2001) recently extended this work demonstrating that gp120-induced neuronal apoptosis is mediated through binding to the CXCR4 chemokine receptors. The in vivo findings of gp120-induced neurotoxicity are also supported by in vitro studies (Lipton et al., 1991). Synergistic neurotoxic effects between gp120 and glutamate had been reported in vitro (Lipton et al., 1991), again suggesting involvement of the NMDA subtype of glutamate receptors in gp120-mediated neuronal damage. Recent studies examined the anatomical specificity within the hippocampal complex of the PPI-disruptive effects of NMDA infusion (0.4 or 0.8 \( \mu g \)) (Swerdlow et al., 2001; Shoemaker et al., 2005). A reduction of PPI in a dose-dependent manner, specifically after NMDA infusion into the ventral subiculum, caudal entorhinal cortex, or CA1 region, suggested the importance of the ventral hippocampal complex in the glutamatergic regulation of PPI (Swerdlow et al., 2001). Thus, in the present study, the findings for a disruption of sensorimotor gating by gp120, indexed by a linear dose-response function, and a leftward peak shift for the high gp120 dose, under the SAL condition, suggests adverse effects on preattentive processes that may be mediated by NMDA mechanisms.

In contrast to an attenuated inhibition response, an enhanced inhibition across ISIs (8–120 ms) was evident on the peak ASR amplitude of the inhibition response in the APO condition as the neonatal gp120 dose increased. Furthermore, the temporal relation between the prepulse stimulus and the startle stimulus, as indexed by the ISI function across PPI trials (0–4000-ms ISIs) suggests a masking or perhaps a mitigation of gp120 neurotoxicity with restoration toward normal by APO (Fig. 4, A–C). The present data indicate that the D\(_1\)/D\(_2\) agonist APO acts on the long-lasting effects in neuronal responses consequent to neonatal gp120 exposure with an alteration of the integrity of the DAergic system.

Recent studies find cumulating evidence that selective populations of cells, such as cells of the DAergic system, are susceptible to viral protein-induced neurotoxicity. Likely targets of HIV-1 virotoxins are dopamine transporters (DATs) in the brain of patients with HAD, specifically in basal ganglia structures like the putamen and ventral striatum (Wang et al., 2004). Studies provide evidence for a decrease on cerebrospinal fluid DA levels in HIV-1 patients and show that this decrease is likely a major contributor to the progression...
of HAD (Berger et al., 1994). It has previously been suggested that DA neurons and DAT may be selectively sensitive to viral toxins (Aksenova et al., 2006; Wallace et al., 2006), and recent human imaging studies find reduced DAT protein levels in HIV-1 patients, especially in those with cognitive and motor deficits (Wang et al., 2004). It is suggested that DA alterations may amplify the neurological manifestation of HIV-1 infection. Selective sensitivity of DA neurons to HIV-1 protein neurotoxins and possible modulation of Tat and gp120 toxicity by drugs that act on the DAergic system has been proposed (Nath et al., 2000a; Aksenov et al., 2006; Aksenova et al., 2006). Thus, APO may serve as a supplement to the lack of DA induced by gp120 and may activate the remaining DA receptors in the damaged system. Despite several reports that D2 agonists robustly disrupt PPI in rats, there is evidence that D1 and D2 receptors work synergistically to modulate PPI in rats (Wan et al., 1996) and that the activation of D2 receptors alone is not sufficient to attenuate PPI of the startle reflex (Martin-Iverson and Else, 2000).

It is not known how APO, as a direct acting stimulant of D1 and D2 receptors, might mitigate gp120-induced neurotoxicity. A recent study suggested disruption of PPI by NMDA activation of the ventral hippocampus in a manner that was dependent on the integrity of infralimbic or cingulate cortical regions that support a D1-mediated regulation of PPI (Shoemaker et al., 2005). A recent in vitro study indicates the importance of D1 receptor-controlled pathways in Tat neurotoxicity in rat cell cultures (Aksenov et al., 2006). It is reported that blockade of D1 receptors in cultured midbrain neurons with the specific D2 antagonist, SCH23390, attenuated cell death caused by HIV-1 Tat + cocaine. The interaction of the D1 damaged system with APO, by activating the remaining D1 receptors, as a direct acting D1 stimulant, might lead to a mitigation of PPI. Our data suggest that interactions between DA and gp120-induced neurotoxicity involve damage to D1 receptors and play an important role in regulation of PPI. The present findings suggest a therapeutic effect of DA agonists, such as L-Dopa effects in Parkinson's disease (Schapira, 2005). However, caution is necessary due to reported effects of an enhanced CNS viral replication, induced CNS vacuolization, and encephalitic lesions with increased DA availability (i.e., administration of selegiline, an agent with DA and neuroprotective properties) (Czub et al., 2004). DA-stimulated transcription through the NF-κB element, specifically in the long terminal repeat, plays an important role in mediating DA responsiveness (Rohr et al., 1999). Thus, the possible implication of these findings for the use of D1 supplements in the gp120 treatment of HIV-1-infected patients needs to be further investigated.

In the present study, long-term gp120 neurotoxicity was detectable in adult rats after neonatal exposure of gp120 at P1 and expressed through the alterations in DAergic system function, as shown by the masking, or perhaps mitigation, of the PPI alterations by APO. The potential underlying mechanism of possible interactions of the D1 damaged system, consequent to gp120 protein exposure, with APO, needs to be further investigated. Deficits in sensorimotor gating in HIV-1 protein-injected rats were documented, as measured by PPI, thus, the investigation of the neural substrates of PPI may yield important information regarding systems involved in HIV-1 pathophysiology.

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References


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