# Neuronal injury in AIDS dementia: Potential treatment with NMDA open-channel blockers and nitric oxide-related species

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ABSTRACT

The neurological manifestations of AIDS include dementia, encountered even in the absence of opportunistic superinfection or malignancy. The AIDS Dementia Complex appears to be associated with several neuropathological abnormalities, including astrogliosis and neuronal injury or loss. How can HIV-1 result in neuronal damage if neurons themselves are only rarely, if ever, infected by the vitus? In vitro experiments from several different laboratiories have lent support to the existence of HIV- and immune-related toxins. In one recently defined pathway to neuronal injury, HIV-infected macrophages/microglia as well as macrophages activated by HIV-1 envelope protein gp120 appear to secrete excitants/neurotoxins. These substances may include arachidonic acid, platelet-activating factor, free radicals (NO.- and $O_2$), glutamate, quinolinate, cysteine, cytokines (TNF-α, IL1-B, IL-6), and as yet unidentified factors emanating from stimulated macrophages and possibly reactive astrocytes. A final common pathway for newonal suscepubility appears to be operative, similar to that observed in stroke, trauma, epilepsy, and several neurodegenerative diseases, including Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis. This mechanism involves excessive activation of N-methyl-D-aspartate (NMDA) receptor-operated channels, with resultant excessive influx of $Ca^{2+}$ leading to neuronal damage, and thus offers hope for future pharmacological intervention.

This chapter reviews two clinically-tolerated NMDA antagonists, memantine and nitroglycerin; (i) Memantine is an open-channel blocker of the NMDA-associated ion channel and a close congener of the anti-viral and anti-parkinsonian drug amantadine. Memantine blocks the effects of escalating levels of excitotoxins to a greater degree than lower (piysiological) levels of these excitatory amino acids, thus sparing to some extent normal neuronal function. (ii) Niuoglycerin acts at a redox modulatory site of the NMDA receptor/channel complex to downregulate its activity. The neuroprotective action of nitroglycerin at this site is mediated by a chemical species related to nitric oxide, but in a higher oxidation state, resulting in transfer of an NO group to a critical cysteine on the NMDA receptor. Because of the clinical safety of these drugs, they have the potential for trials in humans. As the structural basis for redox modulation is further elucidated, it may become possible to design even better redox reactive reagents of chinical value.

To this end, redox modulatory sites of NMDA receptors have begun to be characterized at a molecular level using site-directed mutagenesis of recombinant

subunits (NMDAR1,NMDAR2A-D). Two types of redox modulation can be distinguished. The first type gives rise to a persistent change in the functional activity of the receptor, and we have identified two cysteine residues on the NMDAR1 subunit (#744 and #798) that are responsible for this action. A second site, presumably also a cysteine(s) because <1 mM N-ethylmaleimide can block its effect in native neurons, underlies the other, more transient redox action. It appears to be at this, as yet unidentified, site on the NMDA receptor that the NO group acts, at least in recombinant receptors.

## INTRODUCTION

Approximately a third of adults and half of children with the acquired immunodeficiency syndrome (AIDS) eventually suffer from neurological manifestations, including dysfunction of cognition, movement, and sensation, that are a direct consequence of HIV-1 infection of brain(1,2). These neurological problems occur even in the absence of superinfection with opportunistic pathogens or secondary malignancies(3). Clinical manifestations include difficulty with mental concentration and slowness of hand movements and gait. This malady was initially termed the AIDS Dementia complex by Price and colleagues, but more recently has been placed under the rubric HIV-1 associated cognitive/motor complex. Pathologically, HIV-1 infection in the CNS (or HIV encephalitis) is characterized by widespread reactive astrocytosis, myelin pallor, and infiltration by monocytoid cells, including blood-derived macrophages, resident microglia and multinucleated giant cells. In addition, subsets of neurons display a striking degree of injury, including dendritic pruning and simplification of synaptic contacts, as well as frank cell loss, which may herald the onset of cognitive and motor deficits in affected individuals(3-9), but for another view also see Ref. 10. Such neuronal injury may result in reversible dysfunction rather than inevitable demise. Unlike any other encephalitis studied in recent times, the progressive clinical sequelae occur without direct infection of neurons by HIV-1 or significant autoimmunity induced by virus. Surprisingly, mononuclear phagocytes (brain macrophages, microglia and multinucleated giant cells) in the CNS represent the cell type that is predominantly infected(11). Although infection can occur in astrocytes, it is highly restricted, and perhaps limited to the pediatric population(12,13). An emerging body of evidence strongly supports the idea that activated HIV-1 infected brain macrophages secrete neurotoxins that are largely responsible for the pathological alterations of brain tissue seen following viral infection. These toxins may include HIV-1 proteins (gp120, tat, nef and possibly others) and cell-encoded substances (glutamate-like neurotoxic moleculex, free radicals, cytokines, and eicosanoids) (14-25). The mechanism underlying this indirect form of neuronal injury is related to excessive influx of $Ca^{2+}$ into neurons in response to the noxious factors released from immune activated HIV-infected of gp120-stimulated brain macrophages/microglia (15,26,27).

## BRAIN MACROPHAGE-MEDIATED NEURONAL INJURY: TOXIC SUBSTANCES RELEASED AFTER HIV INFECTION OF gp120 STIMULATION

Production of Cytokines, Quinolinate, Cysteine, PAF, and Free Radicals

A paradox exists between the small numbers of productively HIV-infected brain macrophages/microglia and the severe clinical cognitive and motor deficits that patients with the acquired immunodeficiency syndrome (AIDS) experience. This suggests that some sort of cellular amplification and/or activation is necessary for the generation of viral and cellular toxins that lead to tissue injury and sustained viral infection. Indeed, there is ample evidence for diffuse CNS immunerelated activation in HIV-1 associated neurological impairments(28-30). Moreover, the secretion of neurotoxins by HIV-1 infected macrophages is likely to be regulated by a complex series of intracellular interactions bettween several different brain cell types including mononuclear phagocytes, astrocytes and neurons(27). HIV-infected brain mononuclear phagocytes, especially after immune activation, secrete substances that contribute to neurotoxicity(19,31-33). These include, but are not limited to, eiconsanoids, e.g., arachidonic acid, its metabolites and platelet-activating factor (PAF), proinflammatory cytokines (tumor necrosis factor-alpha [TNF-a] and interleukin-lbeta [IL-1B]), free radicals such as nitric oxide (NO.) and superoxide anion ($O_2^{\cdot-}$), and the glutamate-like agonist, cysteine(19,23-25). Similarly, macrophages activated by HIV-1 envelope protein gp120 release arachidonic acid and its metabolites, TNF-α, IL-β and cysteine. (17,25). Some eicosanoids and free radicals can lead to increased release or decreased re-uptake of glutamate, which can contribute to neuronal damage(22). PAF also elicits neuronal death in in vitro systems by a mechanism probably involving increased neuronal $Ca^{2+}$ and the release of glutamate(23). TNF-α and IL-1β stimulate astrocytosis(34). Chronic immune stimulation of the brain, with widespread CNS (microglial and astroglial) activation, can result from interferon-gamma (IFN-γ) production(28). This immune activation continues the process of nenronal injury initiated by HIV infection and its protein product, gp120. IFN-γ induces production of macrophage PAF(35). and quinolinate, a tryptophan metabolite found in high concentrations in the cerebrospinal fluid of HIV-infected patients with dementia; quinolinate can also act as an glutamate-like agouist to injure neurons(36). Cytokines participate in this cellular network in several additional ways. TNF-α may also increase voltage-dependent calcium currents in neurons(37). In conjunction with IL-1β, IFN-γ can induce nitric oxide synthase (NOS) expression with consequent NO (nitric oxide) production in cultured astrocytes(38). Importantly, most of these facors (cytokines, quinolinate, PAF and products of arachidonic acid metabolism) have been shown to be elvated in brain and/or cerebrospinal fluid of AIDS parients with clinical neurological deficits including dementia(18,23,28,30).

A final common pathway for neuronal susceptibility appears to be operative, similar to that observed in stroke, trauma, epilepsy, neuropathic pain and several neurodegenerative diseases, possibly including Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis. This mechanism involves overactivation of voltage-dependent $Ca^{2+}$ channels and N-methyl-D-aspartate (NMDA) receptor-operated channels, which are also permeable to $Ca^{2+}$ with resultant generation of free radicals(22). Ultimately, the macrophage-synthesized toxins lead to overstimulation of this receptor and increased levels of neuronal $Ca^{2+}$, with consequent release of glutamate. In trun glutamate overexcites neighboring neurons leading to

further increases in intracellular $Ca^{2+}$, neuronal injury, and thus more glutamate release. For many neurons, this pathway to toxicity can be blocked by antagonists of the NMDA receptor(31,39,40). For some neurons, this form of damage can also be ameliorated to some degree by calcium channel antagonists or non-NMDA receptor antagonists, perhaps depending on the repertoire of ion channel types in a specific population of neurons(41). Thus, the elucidation of HIV-1-induced neurotoxins and their mechamism of action(s) offers hope for future pharmacological intervention(27,42).

## HIV-1 COAT PROTEIN gp120 AND NEURONAL INJURY

### gp120 Inhibits Glutamate Re-uptake into Astrocytes via Arachidonic Acid

The coat protein gp120 is thought to exert its predominant deleterious effect on neurons indirectly, via the induction of macrophage toxins, some of which have been elaborated above(43,44). However, direct effects on neurons have not been entirely ruled out. In addition, gp120 may have direct or indirect effects on astrocytes. e.g.,to decrease growth factor production(45). or to inhibit glutamate re-uptake(46). Recently we found that some of these effects may be mediated by arachidonte production of gp120-stimulated macrophages. Arachidonic acid had previously been shown to inhibit re-uptake of excitatory amino acids, such as glutamate, by astrocytes and nerve ending preparations (synaptosomes)(47,48). Since gp120-stimulated macrophages, similar to HIV-infected macrophages, had been shown to produce arachidonate and its metabolites(17,19). it seemed plausible that this arachidonate might affect the re-uptake of excitatory amino acids. In fact, picomolar concentrations of gp120 inhibit excitatory amino acid uptake into cultured astrocytes(16). Purified neonatal rat "astrocyte cultures" also contain approximately 5% monocytoid cells. After depletion of the monocytic cells from these cultures, gp120 no longer inhibits the uptake of excitatory amino acids into astrocytes. This finding implies that this effect of gp120 is predominantly mediated via the monocytic cells in the cluture(16).

One mechanism for the generation of free arachidonic acid is via the activity of the enzyme phospholipase A2. Inhibitors of phospholipase A2, such as aristolochic acid and quinacrine, prevent the decreased re-uptake of excitatory amino acid into astrocytes engendered by gp120. Taken together, these results suggest that a gp120-induced increase in arachidonate production by macrophages is responsible for the inhibition of glutamate uptake into astrocytes(16). Moreover, this mechanism may account at least in part for the observation that gp120 enhances glutamate neurotoxicity in culture(40,49). Arachidonate has also been recently reported to enhance NMDA-evked currents in neurons(50). Therefore arachidonate could contribute to neurotoxicity not only by inhibiting the re-uptake of glutamate but also by increasing the effectiveness of glutamate in evoking NMDA receptor-mediated responses.

### gp120-Stimulated Human Macrophages Release the NMDA Agonist Cysteine

Recently, we have shown that gp120 activation or frank HIV infection enhances

cysteine secretion from human macrophages(25). Cysteine is a known NMDA agonist. (51,52). and could therefore represent at least one of the neurotoxic substances released from gp120-stimulated or HIV-infected macrophages. The release of cysteine by gp120-activated macrophages is greatly attenuated by TNF-α blocking antibodies. Thus, it appears that mactophage secretion of cysteine is mediated by gp120-stimulated production of TNF-α (Yeh and Lipton, manuscript in preparation). These results may serve to link, at least in part the previously recognized pattern of TNF-α elevation in the brains of patrients with AIDS dementia(28,29).

Undoubtedly, other noxious substaneces are released by HIV-infected or gp120--ectivated macrophages, and the identity of these portential neurotoxins are currently under intensive investigation(44). However, the common denominator is that this form of neuroual injury appears to be mediated by excessive increases in intracellular $Ca^{2+}$ levels and can be largely inhibited by NMDA antagonists. (13,31,39,40,44,49,33). For these reasons, studies in AIDS patients with dementia of a clinically-tolerated NMDA antagonist as an adjunctive agent to anti-retroviral therapy has been considered prudent by the AIDS Clinical Trials Group (ACTG) of the NIH. In addition, under some conditions, antagonists of voltage-dependent calcium channels (another mode of entry of $Ca^{2+}$ into neurons)can alleviate neuronal drmage due to excessive activation of NMDA receptors, occurring, for examle, with exposure to gp120(13,34). Along there lines, a phase 1-2 chinical trial for AIDS dementia with the calcium channel antagonist nimodipine was recently completed by the ACTG, and the data indicate that the drug is safe in this population of partients and that a larger trial needs of be performed to assess the possibility of efficacy.

## POTENTLAL CLINICAL UTTLITY OF NMDA ANTAGONISTS FOR AIDS DEMENTI;: OPEN-CHANNEL BLOCKERS AND REDOX CONGENERS OF NTTRIC OXIDE

As detailed elsewhere(22,55), many NMDA antagonists are not climically tolerated, while some others appear to be tolerated by humans at concentrations that are effective neuroprotectants. Several NMDA antagonists have been found to prevent neuronal injuy associated with HIV-infected macrophages, gp120, PAF, cysteine,or quinolinate(27,31,40,44,49,53,56-59). Among these, two of the most promising, becanse of their long experience in patients with other diseases are memanine and nitroglycerin. Memantine blocks the NMDA receptor-associatedtion channel only when it is open. Unlike other NMDA open-channel blockers, such as dizocilpine (MK-801), Memantine does not remain in the channel for an excessively long time, and this kinetic parameter correlates with its safe use in humans for over a dozen years in europe as a treatment for Parkinson's disease(60). Increasing concentrations of glutamate or other NMDA agonists cause NMDA channels to remain open on averge for a greater fraction of time. Under such conditions, an open-channel blocking drug such as memantine has a better chance to enter the channel and block it. Because of this mechanism of action, the untoward effects of greater (pathologic) concentiations of gluramate are prevented to a greater extent than the effects of lower (physiologic) concentrations(22). Moreover, in model systems memantine can ameliorate

gp120-associated nemonal injury(56,57,61).

As discussed above, nitric oxide (NO, where the dot represents one unpaired electron in the outer molecular orbital) con contribute to neuronal darmage, and one of these pathways to neurotoxicity involves the reaction of NO-with $O_2$ to form peroxynitrite (ONÓO-). In contrast, No-can be converted to a chemical state that has just the opposite effect, ie., thar protects neurons from injury due to NMDA receptor-mediated overstimulation. The change in chemical state is dependent on the removal or addition of an electron to NO. This change in the chemical redox state can be influenced by the presence or absence of electron donors such as ascorbate and cysteine. With one less electron, No. becomes nitrosonium ion (NO-), which can react with critical thiol group(s) comprising a redox modulatory site on the NMDA receptor-channel complex to decrease chnnel activity. This reaction can afford neuronal protection from overstimulation of NMDA receptors which would otherwise result in excessive $Ca^{2+}$ influx(52). One such drug that can react with NMDA receptors in a manner resembling nitrosonium is the common vasodilator nitroglycerin(22,52,53,62). Chronic use of nitroglycerin induces toletance to its effects on the cardiovascular system, but the drug still appears to work in the brain to attenunte NMDA receptor-mediated neurotoxicity(55). Nonetheless, the exact dosing regimen has yet to be worked out for the neuroprotective effects of niroglycerin in the brain; therefore, caution has to be exercised before attempting to implement this form of therapy.

In the coming months, as these and other climically-tolerated NMDA antagouists are tested in clinical stadies in as attempt to ameliorate AIDS dementia, we hope to be able to offer our patients better adjunctive therapy to their anti-introviral medicines to treat the cogritive and other neurologic manifestations of AIDS.

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