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Learning impairment following intracerebral administration of the HIV envelope protein gp120 or a VIP antagonist

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The external envelope glycoprotein (gp120) of the human immunodeficiency virus (HIV) has been shown to be toxic to neurons in culture. To further investigate the neurological effects of gp120, the involvement of this protein with the acquisition of spatial discrimination was assessed. Both native and recombinant gp120 were administered into the cerebral ventricles of adult rats and performance was evaluated in the Morris swim maze. Gp120 treatment retarded acquisition after daily administration of 12 ng. The specificity of this impairment was demonstrated in that the performance of animals given the same amount of gp160 from recombinant baculovirus was not different from animals given saline. Vasoactive intestinal peptide (VIP) has been shown to block gp120-induced neurotoxicity in culture and a VIP receptor antagonist has displayed toxic properties to neurons in culture. We show here that this antagonist, which competitively inhibits VIP binding and blocks VIP-mediated functions in cell cultures from the CNS, also produced an impairment of performance. This retardation was attenuated by cotreatment with VIP, supporting the specificity of the observed impairment. Thus, gp120 and the VIP antagonist produced similar retardation of spatial discrimination, suggesting that both may impair memory for spatially related stimulus control.

INTRODUCTION

Cognitive impairment and progressive dementia occur in a high proportion of human immunodeficiency virus (HIV)-infected patients[18,33] and are apparently due to the presence of the virus in the brain[8]. However, in the brain, as in the immune system, extensive damage occurs despite low levels of HIV-infected cells[39,40]. This observation suggests that indirect viral mechanisms, such as the release of a toxic viral product, may be the cause of neurological dysfunction.

The external envelope protein of HIV, gp120, is a viral product with potential neurotoxic activity in the HIV-infected brain. Gp120 is shed from infected cells[38] and previous tissue culture experiments have demonstrated that it is toxic to hippocampal neurons at extremely low (1 pM) concentrations[4]. More recent studies have shown that gp120 also produces cell death in retinal ganglion neurons[7,20] and damages human brain cell aggregates[35]. Administration of purified gp120 in vivo produces neuronal dystrophy in cortical neurons[27] and retards behavioral development[15] in rat neonates.

The neuronal cell death induced by gp120 in hippocampal cultures is potently and completely prevented by vasoactive intestinal peptide (VIP)[4], a neuropeptide associated with neuronal survival[1,2]. Although the mechanism of interaction between VIP and gp120 is still unclear, there are several lines of evidence which indicate that the two substances are functionally related: (1) the distribution of binding sites for gp120 and VIP are similar in the brain[18]; (2) an antibody to CD4, the recognized receptor for HIV, inhibits VIP-mediated chemotaxis in monocytes[37]; (3) a pentapeptide sequence in VIP is homologous to a site on gp120[33]; and (4) treatment of neonatal rat pups with a VIP antagonist retards behavioral development[17] in a manner similar to that induced by gp120[15]. Cotreatment with VIP prevents the antagonist-induced delay[17]. The VIP antagonist, a hybrid molecule of VIP and neurotensin[12], has been previously shown to competitively block VIP receptors in the central nervous system[11], but not on lymphoid cells[13]. Moreover, this antagonist induced neuronal cell death in vitro[10,13].

The current studies were designed to test the possi-

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bility that gp120 would produce cognitive impairment in animals, suggesting a role for this protein in the induction of dementia in HIV-infected individuals. Parallel experiments were carried out to study the effects of VIP on learning. The cognitive effects were assessed in the Morris swim maze[29], previously shown to be sensitive to neurotoxic treatments[30].

MATERIALS AND METHODS

Animals

In addition to unoperated controls, rats (200–250 g, male Sprague–Dawley, Taconic Farms) were stereotaxically implanted, under pentobarbital anesthesia, with stainless-steel cannulas (28-gauge, Plastic Products) placed 1 mm posterior and 1–1.5 mm lateral to bregma, 3.5 mm below the surface of the cranium. Four stainless-steel screws (80-0 0.25 in.) were placed around the cannula and acrylic dental cement was used to anchor the cannula. Placement in the lateral ventricle was subsequently confirmed by injection of dye.

Procedure

Rats were placed in a circular pool, 1.6 m in diameter, 0.38 m deep, equipped with a clear plexiglas column, 3 in wide and high enough to reach just below (approximately 0.5 cm) the surface of the water (22–24°C). During daily testing (13.00–13.30 h) the rat was gently placed into the water at one of 4 starting points. The time lapse from the start until the rat climbed onto the platform was recorded. Visual cues (orientation of lights on the ceiling) remained constant throughout the experiments, other features are as described[29].

Drugs

The rats were allowed to recover from surgery for at least 5 days and then were treated daily with either saline, gp120 (native RF2 strain[36]; low dose = 1.2 ng, high dose = 12 ng), VIP antagonist[12], or VIP (Bachem) plus the antagonist (all 0.7 μg). Additional experiments assessed the effects of 12 ng gp120 (recombinant wild type from HIV_{SF}[14,31]), 12 ng gp160 (recombinant from HIV-1IIIB[20,33]) and 0.7 μg VIP. Two different species of gp120 were used to assess the generality of the phenomenon. Gp160, an inactive glycoprotein which contains the sequences of both gp120 and gp41, was used as a control. Agents were dissolved in sterile saline and given in a volume of 2 μl delivered over 1 min, 4 h before daily testing. The doses used were calculated based on our in vitro experiments[4,11].

Analysis

Statistical significance was determined using repeated-measures analysis of variance with a post-hoc analysis performed using the Fisher's PDS assessed at $P < 0.05$.

RESULTS

As there were no statistical differences between unoperated and saline control groups, the data from both were combined. In these animals the time to find the submerged platform progressively decreased over days of testing (Fig. 1), confirming previous reports of the progressive development of spatial stimulus control. The low dose of $gp120_{RF2}$ (1.2 ng) produced effects similar to those of the control group (Fig. 1). In contrast, a slightly higher dose of $gp120_{RF2}$ (12 ng), impaired the acquisition

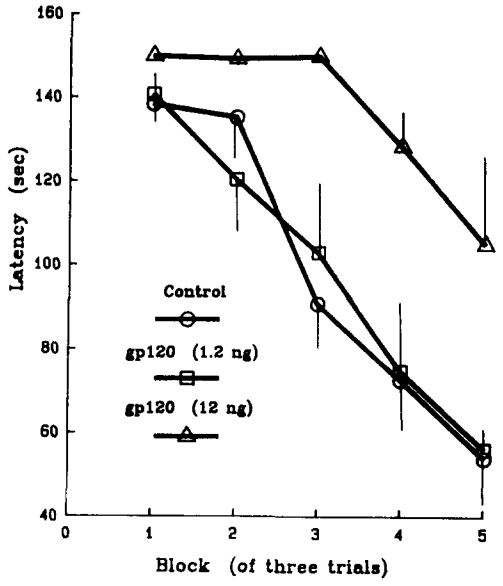

Fig. 1. The effects of a high dose (12 ng) and low dose (1.2 ng) of $gp120_{RF2}$ is compared to controls on the mean time (± S.E.M.) to find a submerged platform in the Morris water maze. Rats were equipped with i.c.v. cannulae and received an injection daily for 1 week prior to initial training, as well as throughout the remainder of the experiment. Beginning 1 week after injection started, rats were placed in a pool and allowed to swim freely until mounting a submerged platform. The abscissa condenses a total of 15 trials run during the study into 5 blocks of 3 trials.

of spatial control, with the greatest effects occurring during the first 9 days of testing (Fig. 1). Both the overall effect of the agent ($F_{5,37} = 2.851$, $P = 0.028$) and trial block ($F_{4,148} = 49.687$, $P < 0.0001$) were significant, whereas the interaction was not. Fig. 2 compares the effects of the same dose of a recombinant $gp120_{SF2}$,

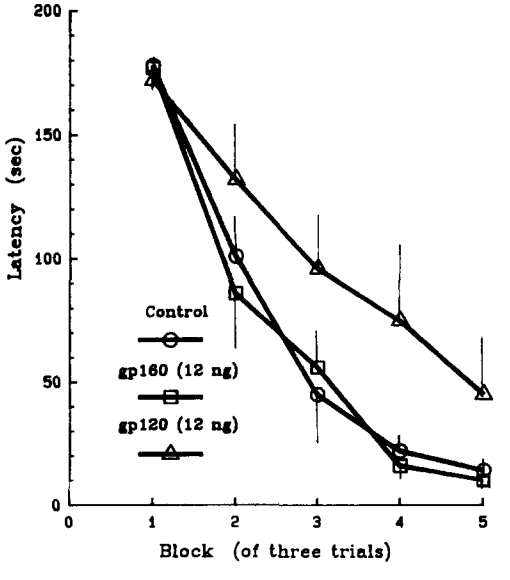

Fig. 2. The effects of recombinant $gp120_{SF2}$ (12 ng), $gp160_{IIIB}$ (12 ng), and saline on the mean time (± S.E.M.) to find a submerged platform in the Morris water maze. Details are as in Fig. 1.

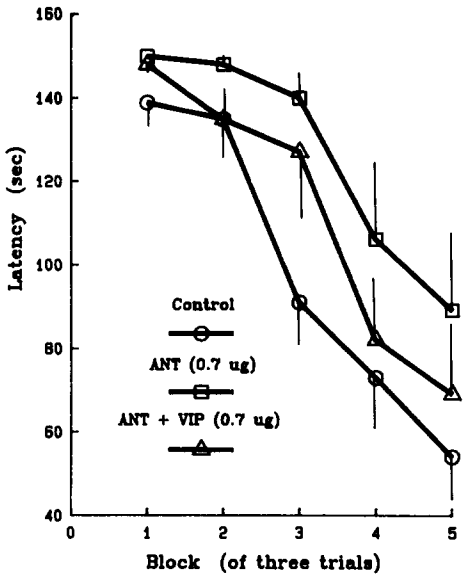

Fig. 3. The effects of the VIP antagonist (0.7 μg), the VIP antagonist in combination with VIP (0.7 μg), and saline on the mean time (± S.E.M.) to find a submerged platform in the Morris water maze. ANT, VIP antagonist. Details are as in Fig. 1.

saline and recombinant $gp160_{IIIB}$, in a systematic replication. The overall effect showed a trend toward significance ($F_{2,14} = 3.422$, $P = 0.06$) while the trial block remained significant ($F_{4,56} = 54.762$, $P < 0.0001$); post-hoc tests (Fisher PSD) confirmed significant differences between saline and gp120, as well as between gp160 and gp120. The VIP antagonist also impaired the acquisition of this performance (Fig. 3). Both the overall effect of the agent ($F_{2,24} = 3.888$, $P = 0.034$) and trial block ($F_{4,98} = 35.429$, $P < 0.0001$) were significant. The VIP antagonist-induced impairment was attenuated by the concurrent administration of VIP. At the concentration used here, VIP alone had no significant effect compared to saline controls (data not shown).

DISCUSSION

Impaired development of spatial control, without a decrement in the ability to eventually navigate to the hidden platform, has been described previously. Both hippocampal lesions[29] and the central administration of an NMDA-specific glutamate receptor neurotoxin[30] produce a pattern of delayed acquisition of place navigation. The effect has been interpreted as one consistent with the loss of limbic-mediated memory for spatially related stimulus control.

We have shown that the i.c.v. administration of a very low dose of the envelope glycoprotein of the autoimmune deficiency syndrome (AIDS) virus, gp120, can retard the acquisition of spatial control in rats. In two independent experiments, using two different gp120 species (native RF2 and recombinant SF2), gp120 was effective in producing this form of memory impairment. In contrast, recombinant gp160 (which contains both gp120 and gp41 sequences and is structurally different from gp120 alone) did not impair performance. Previous studies have shown that gp160 is not toxic to neurons in culture[4].

The results of this study are consistent with the hypothesis that the presence of gp120 in the brains of HIV-infected patients may contribute to their impaired memory and cognitive dysfunction. The infiltration of HIV to the CNS through infected macrophages may result in levels of gp120, or perhaps, a proteolytic fragment of this protein, that can produce comparable functional impairments. The mechanisms by which gp120 induces neural deficits, whether direct or indirect, are unknown and can be related to many similarly affected neuronal pathways[30]. Our results show for the first time that similar learning deficits can be achieved by both gp120 and a VIP antagonist, the latter acting by blocking VIP receptor occupation. It is possible that the gp 120-induced deficits are related to an interference with VIP receptor-mediated function. As outlined previously, there are several lines of evidence which are consistent with the hypothesis that gp120 and VIP interact at some level. Both agents share a common pentapeptide sequence, have similar binding patterns in brain, and VIP prevents the neuronal cell death associated with gp120. VIP has a recognized role in the survival of neurons in CNS cell culture systems[1,2]. In these systems, VIP apparently acts by binding to non-neuronal receptors[10,11] and stimulating the secretion of neuronal survival factors by these cells[2,3]. The actions of gp120, therefore, could be directly or indirectly associated with VIP binding, VIP's secretagogue activity or the biological activity of VIP-induced neurotrophic factors. The VIP antagonist used in these studies has been previously shown to be neurotoxic to spinal cord cells in vitro[11] and to differentiate VIP receptors in the central and peripheral nervous systems[13]. The present study is the first demonstration of the involvement of VIP in learning mechanisms which can be blocked by the VIP antagonist and reversed by VIP.

The recognized receptor for gp120 in lymphoid cells is CD4[23]. Although the existence of a CD4-like receptor in mammalian brain remains controversial, immunocytochemical studies[16] with an anti-CD4 antibody (OKT4) and RNA blot analyses[9] have suggested widespread CD4-like antigen and RNA encoding sequences throughout the brain. Alternatively, several recent studies have shown that HIV may be acting through CD4-independent receptors in the brain. Gp120 binding, HIV-induced fusion, and infectivity of neural cells have been shown to occur in the presence of soluble CD4 or anti-CD4 antibod-

ies. In contrast, these agents inhibited binding[25,26,28] in T cells, suggesting distinct mechanisms for HIV-mediated damage in the brain as compared to the immune system. Regardless of the exact mechanism of gp120's effects, the current results are consistent with the concept that gp120-induced neurotoxicity is related to the impairment of memory.

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