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HIV-1 protein gp120 rapidly impairs memory in chicks by interrupting the glutamate–glutamine cycle

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Abstract

Learning and memory impairments are frequently observed in patients suffering from AIDS Dementia Complex (ADC). These effects have been linked to the presence of gp120, an HIV viral coat glycoprotein. The present study investigated the possibility that gp120 prevents the uptake of extracellular glutamate by astrocytes, leading to an interruption of the glutamate–glutamine cycle and a subsequent impairment of memory. Ten microliters of 10 nM gp120 was bilaterally injected into the region of the intermediate medial mesopallium of day-old chicks at various times before, or after, training using a single-trial passive avoidance task. Gp120 was found to significantly impair memory retention when injected 10–40 min after training. Memory impairments were evident within 5 min of gp120 administration and remained evident 24 h later. Further, the amnestic effect of gp120 could be overcome with glutamine or with precursors of glutamate synthesis, but only weakly by glutamate. These results support the conclusion that the amnestic effect of gp120 is due to an impaired uptake of glutamate by astrocytes and a subsequent interruption of glutamine supply to neurones. The data indicate that the glutamate–glutamine cycle may be a useful therapeutic target in the treatment of ADC.

Keywords: AIDS Dementia Complex; Astrocyte; Chick; Glutamate; Glutamine synthetase; Passive avoidance

1. Introduction

Acquired Immunodeficiency Syndrome (AIDS) Dementia Complex (ADC) is the most common form of dementia in people under the age of 60 years in the USA (Holden, Haughey, Nath, & Geiger, 1999). Up to one third of individuals with AIDS suffer from the symptoms of ADC which include memory deterioration, disturbed sleeping patterns and a loss of fine motor skills (Navia, Jordan, & Price, 1986; Reger, Welsh, Razani, Martin, & Boone, 2002). Long-term neuropathological features of ADC include neuronal degeneration, astrocytosis, myelin pallor, multinucleated giant cells, and elevations in extracellular glutamate (Bagasra et al., 1996; Benos, McPherson, Hahn, Chaikin, & Benveniste, 1994; Brenneman et al., 1988; Holden et al., 1999).

The cognitive and behavioural impairments associated with ADC are thought to be due to either direct effects of the virus (Corasaniti, Bagetta, Rotiroti, & Nistico, 1998; Nuovo et al., 1994; Tardieu, 1999), or its envelope glycoproteins (Kanmogne, Kennedy, & Grammas, 2002; Wang & White, 2000), upon glial cells (Garden et al., 2004). Of particular interest to memory researchers is the Human Immunodeficiency Virus-1 (HIV-1) envelope glycoprotein, gp120. For example, Glowa and colleagues (1992) found that nine days after adult male rats had received intracerebral injections of 12 ng gp120 they exhibited impaired performance in the Morris water maze. Furthermore, Galicia et al. (2000) reported impairments in auditory-cue fear conditioning in rats, following a series of intracerebroventricular injections of gp120 (700 ng over 5 days). Using a similar dosage regime they also reported impaired spatial memory

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in rats using a Barnes maze paradigm (Sanchez-Alavez et al., 2000). Further, Pugh et al. (2000) found that intracerebroventricular injections of gp120 (6, 8 or 10 μg) given to rats immediately after fear conditioning reduced memory recall of the stimulus 48 h later. In the latter two studies, the memory impairments were not observed in rats injected with heat-inactivated gp120.

The impairment of memory caused by gp120 has been suggested to come about as gp120 is known to stimulate NMDA receptors on neurones, potentially leading to neurotoxicity (Gemignani, Paudice, Pittaluga, & Raiteri, 2000; Lipton, Sucher, Kaiser, & Dreyer, 1991). Alternatively, gp120 in targeting astrocytes, causes them to release glutamate and impairs their capacity to take-up glutamate from the extracellular space (Benos et al., 1994). Of particular interest is the finding that exposure of cultured astrocytes to HIV-1, or gp120, reduced the $V_{(max)}$ for glutamate transport in astrocytes by 59%, producing maximal inhibition within 6 h (Wang et al., 2003). This change was correlated with a 40–70% decline in steady state RNA levels of two glutamate transporters: excitatory amino acid transporter 1 and 2 (EAAT1 and EAAT2, respectively), thereby suggesting that gp120 causes transcriptional inhibition of the EAAT glutamate transporter genes (Wang et al., 2003, 2004). EAAT2 (also known at GLT-1) is a key glutamate transporter (Suchak et al., 2003). Located predominantly on astrocytes, pharmacological blockade of this transporter slows the recycling of glutamate by astrocytes. The end result being impairment of synaptic neurotransmission in glutamatergic neurones (Suchak et al., 2003; Turecek & Trussell, 2000; Vorwerk et al., 2000). As glutamatergic neurones are reliant on astrocytes, a gp120-mediated reduction in glutamate uptake by astrocytes may be a significant factor in the cognitive and neuropathological features of ADC.

The relationship between glutamatergic neurones and astrocytes is clearly seen in the glutamate–glutamine cycle. In brief, most of the neuronal glutamate released into the synaptic cleft during neurotransmission is taken up by high affinity transporters on the perisynaptic processes of astrocytes. This glutamate is amidated to glutamine by the astrocytic-specific enzyme, glutamine synthetase (Norenberg & Martinez-Hernandez, 1979). Non-neuroactive glutamine is then released into the extracellular space for uptake by neurones and hydrolysis to glutamate (Pow & Robinson, 1994). Alternatively, neurones can themselves synthesise glutamate by amidation of α-ketoglutarate (an intermediate of the tricarboxylic acid cycle), followed by the addition of an amino acid group to form the glutamate side chain. Alanine, a derivative of lactate which is also supplied to neurones by astrocytes (Hertz, Dringen, Schousboe, & Robinson, 1999) is a suitable source of this amino acid.

Importantly, glutamate is the main excitatory neurotransmitter used in the pathways associated with memory in mammals and birds (Ng et al., 1997). Hence, unimpaired uptake and recycling of glutamate is a prerequisite for memory consolidation. In chicks for example, the blockade of glutamate uptake by L-aspartic acid β-hydroxamate administered 5 min before learning prevented memory consolidation for an aversive stimulus (Gibbs, Hertz, & Ng, 2004). Furthermore, inhibition of astrocytic glutamine synthetase by methionine sulfoximine (MSO) abolished memory retention from 20 min post-training (Gibbs et al., 1996). Moreover, the amnestic effects of MSO were prevented by co-administration with either L-glutamine (10 mM), monosodium glutamate (4 mM) or a cocktail of the glutamate precursors α-ketoglutarate (5 mM), and alanine (5 mM) (Gibbs et al., 1996). These studies demonstrate the importance of glutamate uptake and its subsequent amidation to glutamine in consolidation of the memory trace.

Since gp120 has been reported to impair the uptake of glutamate by astrocytes, it is possible that the amnestic effect of this glycoprotein is due to an interruption of the glutamate–glutamine cycle. This study examined whether administration of gp120 impaired the performance of chicks on a single-trial passive avoidance-learning task, and if so, whether such impairments could be overcome by co-administration of glutamine, glutamate or a cocktail of α-ketoglutarate and alanine.

2. Materials and methods

2.1. Animals

Day-old black Australorp x white Leghorn cockerels were obtained from a local hatchery on the morning of each experimental day. Birds were randomly paired and housed in wooden pens (20 cm × 25 cm × 20 cm). All experimental procedures were carried out with the chicks in pairs to prevent isolation stress (De Vaus, Gibbs, & Ng, 1980). Room temperature was maintained at 25–30 °C by single 15 W white light globes situated above each pen. Chick food was available ad libitum.

2.2. Drug preparation

Recombinant gp120 was donated by AIDS Reagent, USA. Gp120 was diluted in a phosphate buffer solution (PBS) to yield 1 μM stock solutions and was stored in 20 μL aliquots at −80 °C until use. On days of experimentation the gp120 stock solutions were diluted to the required concentration in sterile saline (154 mM NaCl; Sigma Co.) and stored in ice until 5 min before administration. Control solutions of heat-inactivated gp120 were obtained by storing the required concentration of active gp120 solution at room temperature for 5 days (Holden et al., 1999). In addition to inactivated gp120, the saline vehicle was used as a control in some experiments. In the challenge studies, a drug cocktail was injected consisting of active gp120 with either of 4 mM L-glutamic acid (Sigma Co.), 10 mM L-glutamine (Sigma Co.) or a combination of 5 mM α-ketoglutarate (Sigma Co.) and 5 mM alanine (Sigma Co.). Drugs were prepared fresh on the morning of each experimental day.

A 10 μl volume of each drug, or drug cocktail, was delivered freehand into the region of the intermediate medial mesopallium of both cerebral hemispheres using a Hamilton syringe with repeating dispenser. This region is critical to memory formation for this task (Rose & Csillag, 1985). Accuracy of injections was guided by random histological checks of needle tracks in the brains of injected chicks. However, there is bound to have been some diffusion of the drugs beyond this region, so we cannot exclude the possibility that other regions involved in memory, such as the medial striatum, were also affected.

Drugs were injected either before or after learning, depending upon the aim of the experiment.