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Voltage-gated Potassium Channels in Human Immunodeficiency Virus Type-1 (HIV-1)-associated Neurocognitive Disorders

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Abstract

Human immunodeficiency virus type-1 (HIV-1)-associated dementia (HAD), a severe form of HIV-associated neurocognitive disorders (HAND), describes the cognitive impairments and behavioral disturbances which afflict many HIV-infected individuals. Although the precise mechanism leading to HAD is incompletely understood, it is commonly accepted its progression involves a critical mass of infected and activated mononuclear phagocytes (brain perivascular macrophages and microglia) releasing immune and viral products in the brain. These cellular and viral products induce neuronal dysfunction and injury via various signaling pathways. Emerging evidence indicates voltage-gated potassium (K_v) channels, key regulators of cell excitability and animal behavior (learning and memory), are involved in the pathogenesis of HAD/HAND. Here we survey the literature and find that HAD-related alterations in cellular and viral products can increase neuronal K_v channel activity, leading to neuronal dysfunction and cognitive deficits. Thus, neuronal K_v channels may be a new target in the effort to develop therapies for HAD and perhaps other inflammatory neurodegenerative disorders.

Keywords

voltage-gated K^+ channels; HIV-1-associated dementia; mononuclear phagocytes; cytokines; long-term potentiation; learning and memory

Introduction

In the highly active antiretroviral therapy (HAART) era, persistent low-level viral infection of mononuclear phagocytes (MP; perivascular macrophages and microglia) has supplanted high-level productive human immunodeficiency virus (HIV) replication as the pivotal

mechanism for the pathogenesis of HIV-1-associated dementia (HAD), a severe form of HIV-1-associated neurocognitive disorders (HAND; Antinori et al. 2007). HAD describes the cognitive deficits, motor disturbances, and behavioral abnormalities often observed in HIV-infected individuals (for reviews see (Kaul et al. 2001; McArthur et al. 2003; Gonzalez-Scarano and Martin-Garcia 2005; Kramer-Hammerle et al. 2005; Verani et al. 2005)). Although the incidence of HAD has decreased from an estimated 30% to ~10% in the era of HAART, it remains a significant complication of HIV-1 infection as patients with acquired immunodeficiency syndrome (AIDS) live longer, antiretroviral drugs remain unable to effectively cross the blood brain barrier, and HIV-1 resistance grows due to viral strain mutation. It is widely believed HAD cognitive impairments are associated with the number of immune competent and HIV-1-infected MP (Glass et al. 1995), diminished synaptic and dendritic density (Masliah et al. 1997; Everall et al. 1999), and selective neuronal loss (Masliah et al. 1992; Fox et al. 1997). However, not all HAD patients exhibit profound neuronal loss (Seilhean et al. 1993) and improvements in cognitive function have been observed after HAART (Ferrando et al. 1998; Gendelman et al. 1998; Tozzi et al. 1999; Robertson et al. 2004). This suggests that cognitive decline may result as much from neuronal dysfunction as from neuronal loss, an idea supported by experimental results showing alterations in cell layer volume (Sa et al. 2000) and dendritic morphology (Masliah et al. 1997) correlate with HAD (Gray et al. 2001).

Unfortunately, despite more than two decades of investigation, the precise mechanisms of HAD pathogenesis are not fully understood. However, accumulating evidence indicates neuronal voltage-gated potassium (K^+) channels (K_v) play a role in memory processes (Giese et al. 1998, 2001; Solntseva et al. 2003) and acquired neuronal channelopathies in HAD (Gelman et al. 2004). Moreover, K_v channel antagonists have been found to improve animal learning and memory (Andreani et al. 2000; Keblesh and Xiong, unpublished data). Therefore, to understand the role K_v channels might play in HAD pathogenesis, here we survey the literature to clarify the link between increased neuronal K^+ current and cognitive dysfunction and to ascertain whether the alterations in the levels of HAD-related cellular or viral products can increase neuronal K_v channel-conducted currents.

K_v channels, neuronal excitability, and cognition

K_v channels are present in every known excitable cell and are ubiquitous in the central nervous system (CNS). Despite their diversity and modulatory sensitivity, K_v channels can be categorized based on their voltage sensitivity (low- or high-voltage activated) and inactivation tendencies (delayed rectifier (I_K) or A-type (I_A); Dodson and Forsythe 2004). The large number of different K_v channel genes and possible arrangements of the four α -helical subunits, which form homo- or heteromeric tetramers to create a functional channel (Coetzee et al. 1999), reveal a built-in diversity allowing for an array of functional capabilities to suit the needs of each excitable cell. It is well known K_v channels play a crucial role in the generation of electrical activity in a neuron including, but not limited to, setting and stabilizing the resting membrane potential, repolarizing action potentials (APs), and controlling the discharge frequency through the regulation of interspike intervals. For example, the ability of a neuron to fire APs and the shape of AP waveforms is mainly regulated by K_v channels. Thus, K_v channels directly influence neuronal excitability. The functions of K_v channels can be modulated by a number of factors, including membrane potential, redox potential, posttranslational modification, and a plethora of organic molecules and peptides (Hille 2001; Birnbaum et al. 2004). K_v channel functions can also be modulated by other bioactive molecules such as proinflammatory cytokines. Such modulations of K_v channel functions could lead to an altered excitability, resulting in neuronal dysfunction.

K_v channels and learning and memory

It has been proposed the number and pattern of APs encode information (Rieke et al. 1997). This suggests a change in K_v channel activity may alter information processing (e.g., learning and memory). Indeed, recent genetic targeting studies indicate K_v channels have a great importance in memory processes (Giese et al. 1998, 2001; Solntseva et al. 2003). It has been demonstrated in different model systems K⁺ current decreases during learning and antagonists of K_v channels can improve learning and memory (Andreani et al. 2000). The dysfunction or alteration of K_v channels is therefore believed to be an important link in the mechanisms of memory disturbance (Solntseva et al. 2003).

Regardless of the type of K_v channel, the negative equilibrium potential of K⁺ relative to the action potential threshold lends an essentially inhibitory nature to K⁺ currents. Several studies have shown a decreased K⁺ channel current, whether by antagonist or mutation, corresponds with improved memory and long-term potentiation (LTP), while an increased K⁺ current corresponds to the learning and memory deficiencies (Ghelardini et al. 1998; Alkon 1999; Solntseva et al. 2003).

The necessity of healthy K⁺ channel function to learning and memory processes can be seen in the vital roles they play in each segment of the neuron (Dodson and Forsythe 2004). In the distal dendrites, K_v4 A-type channels have been proposed as a convergence point for the functional integration of LTP-related signal transduction pathways (Olds et al. 1989; Alkon et al. 1998; Dineley et al. 2001; Birnbaum et al. 2004). Increasing the dendritic A-type K⁺ current decreases back-propagating AP (bp-AP) amplitudes and lowers the probability of LTP induction (Watanabe et al. 2002). Alternatively, downregulation of the K_v4 channel subtype has been demonstrated to enhance LTP, increase excitatory postsynaptic potential-spike potentiation (Frick et al. 2004), and improve learning and memory behaviors (Lilliehook et al. 2003). By regulating bp-APs (Hoffman et al. 1997), dendritic K_v4 channels determine the depolarization sensed by postsynaptic *N*-methyl-D-aspartate (NMDA) receptors and thereby play a crucial role in the networking properties underlying learning and memory (Paulsen and Sejnowski 2000; Johnston et al. 2003; Birnbaum et al. 2004).

In the proximal dendrites and soma, recent studies have demonstrated K_v1.1/K_vβ1.1 contributes a somatodendritic A-type current (Giese et al. 1998). Deletion of the K_vβ1.1 subunit gene results in reduced I_A amplitude, increased neuronal excitability, improved Morris water maze performance, and a decreased threshold for the induction of LTP (Giese et al. 1998; Murphy et al. 2004). K_vβ1.1-deficient mice also exhibit reductions in both frequency-dependent spike broadening and the slow after hyperpolarization, modulation of which has been proposed as a learning and memory mechanism (Giese et al. 1998, 2001; Murphy et al. 2004). Meanwhile, clusters of K_v2.1 channels account for the somatodendritic I_K (Murakoshi and Trimmer 1999; Du et al. 2000; Pal et al. 2003) and are necessary for regulating both intrinsic and neuronal excitability during high-frequency stimulation (Du et al. 2000; Misonou et al. 2005). Excitotoxic Ca²⁺-dependent dephosphorylation of K_v2.1 channels causes cluster dispersion (Misonou et al. 2004; Misonou et al. 2005), shifts the K_v2.1 activation curve to more hyperpolarized potentials, and increases the open channel probability (Murakoshi et al. 1997). K_v2.1 dephosphorylation also results in a dendritic beading correlated with diminished LTP (Misonou et al. 2004). Rephosphorylation of K_v2.1 prevents dendritic injury and restores function, demonstrating a strong link to potassium channel activity (Misonou et al. 2004).

Potassium channels also function to faithfully transmit APs in axons and nerve terminals (Dodson and Forsythe 2004). An A-type K⁺ channel antagonist (likely blocking K_v1.4 or K_v1.1/K_vβ1.1) returned function in induced axonal propagation failure experiments,

suggesting a role in gating signal propagation (Debanne et al. 1997, 1999). Meanwhile, noninactivating low-voltage-activated axonal K^+ currents suppressed hyperexcitability and reduced aberrant firing (David et al. 1995; Tan and Llano 1999; Zhou et al. 1999). Outward K^+ current was also detected in the relatively inaccessible presynaptic compartments of several mammalian cell types (Jackson et al. 1991; Thorn et al. 1991; Forsythe 1994). A noninactivating low-voltage-activated current, likely through $K_v1.1$ or $K_v1.2$, has been found to suppress hyperexcitability, raise the AP threshold, and oppose the aberrant firing of APs (Dodson et al. 2003), while high-voltage-activated channels are thought to keep the AP duration short and limit transmitter release (Sekirnjak et al. 1997; Southan and Robertson 2000; Elezgarai et al. 2003). In addition, the cumulative inactivation of a transient outward current (likely $K_v1.4$) upon repeated depolarization broadens the AP and enhances transmitter release (Jackson et al. 1991; Thorn et al. 1991). While more direct research is necessary, the role of these channels in axonal and terminal neuronal function has clear implications for learning and memory.

K_v channels and apoptosis

The role of potassium channel function in apoptosis has also recently been investigated in several cell types, including smooth muscle cells, eosinophils, enterocytes, lymphocytes, thymocytes, T cells, and neurons, among others (for reviews see (Yu 2003; Burg et al. 2006)). Using various methods to induce apoptosis in these different cell types, increased K^+ efflux and intracellular K^+ depletion were found to mediate apoptotic volume decrease and depression of apoptotic effectors, resulting in cell shrinkage, caspase activation, cytochrome *c* release, endonuclease activation, DNA fragmentation, and eventual apoptosis. In cultured (neo)cortical neurons, apoptosis was accompanied by increased delayed rectifier K^+ current, and both potassium channel blockers and high K^+ medium were found to be protective against cytochrome *c* release, caspase-3 activation, DNA damage, and apoptosis (Yu et al. 1997; Wang et al. 2000; McLaughlin et al. 2001; Xiao et al. 2002). While well-regulated apoptosis serves to maintain tissue homeostasis, the dysregulation of apoptosis could lead to premature cell death (i.e., neurodegeneration).

K_v channels and HAD pathogenesis

HIV-1-infected patients who were followed prospectively and exhibited neuropsychiatric decline before death demonstrated increased MP numbers in the brain (Glass et al. 1995), decreased synaptic and dendritic density (Masliah et al. 1997; Everall et al. 1999) and selective neuronal loss (Masliah et al. 1992; Fox et al. 1997) on subsequent neuropathological evaluation. The distribution of damaged neurons has been shown to be closely associated with markers of MP activation, especially within the subcortical deep gray structures (Adle-Biassette et al. 1999), suggesting soluble factors released from HIV-1-infected and/or immune-activated MPs may be the source of neuronal dysfunction. It is now widely believed these soluble factors, including, but not limited to, cytokines, chemokines, excitatory amino acids, arachidonic acid (AA) metabolites, nitric oxide, and viral proteins (HIV-1 glycoprotein 120 (gp120), transactivator of transcription (Tat) and negative factor (Nef)), are the primary cause of neuronal dysfunction or injury (Lipton 1991; Genis et al. 1992; Tyor et al. 1992; Gelbard et al. 1994; Toggas et al. 1994; Bukrinsky et al. 1995; Nottet and Gendelman 1995; Xiong et al. 2000; Yeh et al. 2000; Kaul et al. 2001; Carlson et al. 2004). Whereas many studies have shown these soluble factors induce neuronal dysfunction via different signaling pathways, emerging evidence indicates they can mediate neuronal dysfunction and/or injury through the activation of neuronal K_v channels, resulting in neurocognitive dysfunction. The effects of soluble factors on K_v channels are summarized in Fig. 1.

Tumor necrosis factor- α (TNF- α)

One such substance is the proinflammatory cytokine tumor necrosis factor- α (TNF- α). Reverse transcription-polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), and biological assays demonstrate elevated macrophage synthesis of TNF- α in HIV-infected macrophage/astroglia cocultures (Epstein and Gendelman 1993; Gelbard et al. 1994). In addition, HIV-1 envelope protein gp120 has been found to upregulate TNF- α expression in cultured human macrophages (Yeung et al. 1995), while HIV-1 Tat protein stimulates the release of TNF- α in rat microglial cultures (Nicolini et al. 2001). Importantly, RT-PCR of homogenized brain tissue collected during autopsies of patients, ranging from seronegative to those diagnosed with HAD, reveals an increased expression of TNF- α mRNA correlating with symptom severity (Glass et al. 1993). The primary accepted effect of elevated TNF- α in HAD neuropathology is to promote the entry of HIV-infected cells into the CNS by increasing the permeability of blood brain barrier (Fiala et al. 1997), inducing the expression of cell adhesion molecules, and upregulating monocyte chemoattractant protein-1 (MCP-1; Nottet 2005). An additional generally accepted effect of TNF- α is the robust inhibition of high-affinity glutamate scavenging in glial cells, which can contribute to excessive extracellular glutamate accumulation and resultant neurotoxicity (Fine et al. 1996; Zou and Crews 2005). Of relevance here, however, are whole-cell patch-clamp recordings demonstrating an increase in A-type K^+ current when recombinant human TNF- α is applied to cultured embryonic rat cerebral cortex neurons, possibly through protein kinase C signal transduction pathways (Houzen et al. 1997). Although it has been suggested these currents may have neuroprotective effects against overexcitation, NMDA currents were not activated at this recombinant human TNF (rhTNF)- α concentration and incubation duration, and the A-type K^+ current was found to decrease after extended rhTNF- α exposure (Houzen et al. 1997). This suggests an early physiological role for TNF- α in neuronal dysfunction through an increased K_v current, possibly followed by a glutamate-related toxicity to be discussed later (Fine et al. 1996; Bezzi et al. 2001). Furthermore, electrophysiology experiments demonstrate relatively short duration TNF- α applications inhibit LTP in the CA1 region of rat hippocampal slices in a dose-dependent manner (Tancredi et al. 1992), while a low-dose application can completely abolish LTP in the rat dentate gyrus (Cunningham et al. 1996). Therefore, even though the competing neurodegenerative and neuroprotective roles of TNF- α are complex and debatable (Brabers and Nottet 2006), TNF- α related increases in K_v current may yet prove relevant to HAD learning and memory deficits.

Interleukin-1 β

Similarly, immunocytochemistry performed on brain tissue collected at autopsy reveals a significant increase in a second proinflammatory cytokine, interleukin-1 β (IL-1 β), in HAD patients compared with nondemented seropositive patients (Zhao et al. 2001). RT-PCR, ELISA, and biological assays have also demonstrated IL-1 β levels are elevated in HIV-infected macrophage/astroglia cocultures (Genis et al. 1992; Epstein and Gendelman 1993). Moreover, the application of HIV-1 Tat protein to rat microglial cultures (Nicolini et al. 2001) and the intracerebroventricular injection of HIV-1 envelope protein gp120 into rats (Barak et al. 2002) have both been shown to stimulate IL-1 β production. Notably, the behavioral abnormalities associated with gp120 injections were attenuated with IL-1 antagonist pretreatment. The effect of increased IL-1 β is thought to be similar to that of TNF- α , inducing cell adhesion molecule expression and upregulating MCP-1 (Brabers and Nottet 2006), while there is also evidence IL-1 β may play a facilitating role in HAD-related excitotoxic NMDAR-mediated Ca^{2+} influxes (Wang et al. 1999). Importantly, however, the application of IL-1 β has also been shown by intracellular recording to increase outward conductance and synaptic inhibition during the stimulation of hippocampal CA1 pyramidal neurons, likely effecting neuronal plasticity (Zeise et al. 1992). In *Helix pomatia*, two-

microelectrode voltage clamp demonstrated IL-1 increases both the transient and delayed rectifier K^+ currents in a voltage- and dose-dependent manner (Szucs et al. 1992). This perhaps explains why field recordings in the rat dentate gyrus exhibited diminished long-term potentiation after pretreatment with IL-1 β (Cunningham et al. 1996) and in the CA1 with superfusion of IL-1 β (Bellinger et al. 1993). Also similar to TNF α , IL-1 β is thought to have both neurodegenerative and neuroprotective properties (Brabers and Nottet 2006), suggesting that the most beneficial treatments may be those which can isolate and target downstream neurotoxic effects.

Arachidonic acid and its metabolites

Arachidonic acid is another key molecule which is known to effect potassium channels and is altered in HAD. Analysis following high performance liquid chromatography separation of HIV-1 infected monocytes and human glia coculture media has demonstrated AA is converted to its metabolites at an abnormally high rate during HIV-1-infected macrophage-astroglia interactions (Genis et al. 1992). The significance of this lies in understanding the differential effects of AA and AA metabolites on potassium current. Early research has shown that AA is released during high-frequency input (Williams et al. 1989, 1993) via NMDA receptor activation (Dumuis et al. 1988) and is an essential messenger in long-term potentiation (Nishizaki et al. 1999). Recent research reveals AA suppresses somatodendritic transient potassium current in rat CA1 pyramidal cells, which facilitates action potential discharges by increasing the postsynaptic response to stimulation and enhancing the summation process, resulting in a lowered threshold for LTP (Ramakers and Storm 2002). In contrast, the metabolic products of AA have been found to increase both the open channel probability (Piomelli et al. 1987) and the current (Zona et al. 1993) of sustained potassium channels in cultured Aplysia sensory cells and cultured rat neocortical neurons, respectively, thereby dampening excitability. The emerging theme is one of a necessary balance, which enables reliable synaptic transmission without compromising stability (Colbert and Pan 1999). Under HAD conditions, the increased conversion of AA to its metabolites may reduce AA suppression of transient K^+ current, while at the same time increasing the AA metabolite enhancement of sustained K^+ current, possibly leading to a channelopathic increase of both types.

Brain-derived neurotrophic factor

In addition to the increased levels of TNF α , IL-1 β , and AA metabolites, HAD models have uncovered an associated decrease in brain-derived neurotrophic factor (BDNF). While BDNF is known to be crucial to the processes of neurogenesis, neuronal development, and neuronal survival, more recent studies also demonstrate it to be essential for long-term potentiation and other forms of activity-dependent synaptic plasticity (Korte et al. 1996; Hartmann et al. 2001; Zakharenko et al. 2003). Interestingly, BDNF was found to reduce gp120-related neurotoxicity (Nosheny et al. 2005); unfortunately, after striatal injections of HIV-1 envelope protein gp120 into rats, immunoassays revealed a significant reduction in BDNF expression (Nosheny et al. 2004). Of particular relevance here, however, are studies which demonstrate BDNF has the ability to downregulate $K_v1.2$, $K_v1.4$, and $K_v4.2$ in dorsal root ganglia (Park et al. 2003). This suggests BDNF may exert its positive effect in HAD models via the downregulation of A-type potassium channel genes and their associated currents. Moreover, it allows for the possibility that a dramatic decrease in the concentration of this gene regulator in the HAD condition could result in an increase in A-type potassium channel gene expression, accompanied by an increase in transient K^+ current.

Glutamate

As alluded to previously, recent evidence suggests that enhanced glutaminase activity in HAD could result in an overabundance of glutamate (Erdmann et al. 2006). This would

likely result in the pathological activation of NMDA currents and associated increases in Ca^{2+} influx. Of interest to us, however, is that an increase in glutamate may also have an effect on potassium channel function. Upon treatment with glutamate, neuronal $\text{K}_v2.1$ channels have been demonstrated to undergo Ca^{2+} -dependent dephosphorylation (Misonou et al. 2004). Consequently, $\text{K}_v2.1$ channel clusters are dispersed and their activation curves shifted, such that the open channel probability and current are increased (Murakoshi et al. 1997). Moreover, this dephosphorylation also causes a dendritic beading which has been linked to potassium channel related LTP diminishment (Gelbard et al. 1994; Misonou et al. 2004; Bellizzi et al. 2005). Importantly, increased K^+ efflux through $\text{K}_v2.1$ channels has also been demonstrated to result in a loss of cell volume associated with apoptosis (Pal et al. 2003). Taken together, these results are consistent with the learning and memory deficiencies observed in HAD patients, the loss of cell layer volume due to atrophy in the CA1 region of AIDS patients (Sa et al. 2000), as well as late-stage limited cell loss (Masliah et al. 1992; Everall et al. 1993).

HIV-1 protein Nef

In addition to the altered levels of the aforementioned cellular factors, the presence of the viral protein Nef has been detected in the sera of AIDS patients (Deacon et al. 1995) and in brain tissue recovered after autopsy of HIV-1-infected patients with neuronal damage (Ranki et al. 1995). Nef is a soluble, viral encoded accessory protein which is essential for efficient viral replication and pathogenesis (Kestler et al. 1991). Intriguingly, Nef shares functional sequences with scorpion peptides known to interact with potassium channels (Garry et al. 1991; Werner et al. 1991). Moreover, patch-clamp experiments have shown Nef reversibly increases the total K^+ current in chick dorsal root ganglions (Werner et al. 1991), allowing for the possibility of a direct link between HIV-1-encoded viral protein and increased potassium channel current. Notably, the finding that the direct application of Nef exhibits neurotoxic effects on cultured human neurons (Trillo-Pazos et al. 2000), possibly through excessive K^+ efflux, would seem to further support this view.

Other pathways

All the products and pathways described so far suggest an HAD-related increase in K^+ current. However, just as some pathways altered in HAD are neutral or neuroprotective, some may lead to a decreased potassium channel current. For instance, gene expression profiling studies show decreased expression of two probable voltage-driven K^+ channels, which could result in decreased K_v current (Gelman et al. 2004). Similarly, recent studies suggest extracellular response kinases (ERK1/2) are activated in HAD (Meucci et al. 1998), which could lead to ERK phosphorylation of $\text{K}_v4.2$ channels (Adams et al. 2000) and a subsequent reduction in transient A-type current (Schrader et al. 2006). Notably, inhibition using ERK (p42/p44 mitogen-activated protein kinase) antagonists has been shown to shift the dendritic A-type K^+ current activation curves to more hyperpolarized potentials and lower the probability of LTP induction (Watanabe et al. 2002), confirming this pathway can indeed regulate potassium current.

Macrophage-conditioned media

It is clear the aforementioned cellular and viral products induce alteration of neuronal K^+ current. The alterations produced by each of these individual products may be additive and ultimately lead to neuronal dysfunction or damage. Recent studies from our laboratory have revealed immune-activated macrophage-conditioned media (MCM; MCM+) increases both the transient A-type (Hu and Xiong, unpublished results) and delayed rectifier K^+ current (I_K) in cultured rat hippocampal neurons (Fig. 2a). The MCM-associated increase of I_K was significantly blocked by tetraethylammonium (TEA), a K_v channel antagonist (Fig. 2b). We have also examined the biological significance of MCM-induced enhancement of I_K by

testing neuronal viability in the presence and absence of TEA. Utilizing propidium iodide (PI) and 4'-6-diamidino-2-phenylindole (DAPI) fluorescent staining, we found the MCM-mediated K_v channel current increases were correlated with diminished cell survival, which was attenuated by TEA (Fig. 2c, d).

4-Aminopyridine ameliorates HIV-1-associated neuronal dysfunction

As a change of K^+ channel activity may alter information processing (i.e. learning and memory), the MCM-mediated increase of K^+ current may contribute to the cognitive dysfunction seen in patients with HAD. Previously, we demonstrated severe combined immunodeficient (SCID) mice injected with HIV-1-infected monocyte-derived macrophages in the basal ganglia exhibit impaired performance in Morris water maze tests (Zink et al. 2002; Anderson et al. 2003). We have also now demonstrated MCM+ enhances neuronal outward currents, namely I_A and I_K . This MCM-associated enhancement of outward K^+ currents may represent a link between neuronal dysfunction and cognitive decline. Thus, we supposed a systematic administration of a K^+ channel antagonist might lead to the improvement of learning and memory in SCID mice with HIV-1 encephalitis, a mouse model recapitulating many aspects of HIV disease in human and consistently exhibiting impaired LTP in the hippocampus (Zink et al. 2002; Anderson et al. 2003). We found the systemic administration of 4-aminopyridine (4-AP) significantly improved animal performance in radial-arm water maze tests (Keblesh and Xiong, unpublished data). Furthermore, these behavioral results were confirmed by, and correlated with, an electrophysiological study of LTP in the CA1 of the hippocampus, giving further support to the view that neuronal dysfunction in HAD is related to a chronic increase in potassium channel current.

Abnormal electroencephalography in HAD

Several distinct types of K_v channels are expressed in the brain and serve a wide range of functions. They mediate rapid spike repolarization and fast after hyperpolarization and may significantly influence dendritic action potential back propagation and responsiveness of a neuron to synaptic input (Schwindt et al. 1988; Pongs 1999; Womack and Khodakhah 2002). The K_v channel dysfunction may elucidate how abnormal electrical current output from brain cortex is produced in HAD, which can be recorded by electroencephalography (EEG) using scalp electrodes. It has been shown that an increase in the amplitude of α and θ waves of EEG can be recorded in HAD patients (Baldeweg and Gruzelier 1997; Polich et al. 2000; Jeong et al. 2001) and a left/bilateral temporooccipital focal slowing on EEG was described (Mehling et al. 2008). As EEG wave oscillations originate from feedback loops between brain neocortex and subcortical regions such as the basal ganglia (Llinas et al. 1999; Gelman et al. 2004), the abnormal EEG recorded in HAD patients may reflect the underlying theory of the subcortical dementia (Gelman et al. 2004). In such a scenario, subcortical disturbance induced by HIV-1 and HIV-1-infected mononuclear phagocytes may result in cortical neuronal channelopathies (Gelman et al. 2004). Thus, the recognition of acquired neuronal channelopathies in HIV-1-infected brain initiates a new era in understanding the molecular pathophysiology of HAD.

Summary

HAD today remains a significant consequence of HIV infection for many patients. The mechanism of HAD is incompletely understood; however, mounting evidence suggests that a reversible neuronal dysfunction prior to cell loss may play a significant role in advancing HAD. Given the essential nature of K^+ channel function to neuronal processes, we reviewed the literature to determine if there was a connection between HAD and K^+ channel dysfunction. The evidence gathered suggests several HAD related cellular and viral products

do indeed increase outward K^+ current. Moreover, increased outward K^+ current, disturbed learning and memory processes, and diminished neuronal survival were confirmed in our own laboratory in an animal model of HAD. Up until now, much of the research emphasis has been focused on slowing viral replication rather than targeting the functional consequences of HAD for the neuron. While many questions remain to be answered, knowledge of the K^+ channel dysfunction which may underlie HAD and other neurological conditions can serve as a clear starting point for the development of adjunctive therapies which specifically target the disrupted neuronal function.

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Fig. 1.

Effects of soluble factors on the voltage-gated K^+ currents of neurons. This diagram summarizes the effects of identified cytokines, arachidonic acid, glutamate, viral protein, and unidentified factors in macrophage-conditioned media. I_A , A-type K^+ current; I_K , delayed rectifier K^+ current. *Unpublished data

Fig. 2.

Macrophage-conditioned media induces neuronal injury through the activation of neuronal K_v channels. **a** An example of the outward delayed rectifier K^+ current recorded from a cultured rat hippocampal neuron in the presence of a 5-mM 4-AP. The cell was held at -60 mV, stepped to -40 mV, and followed by the steps of 10-mV increments. Bath application of the lipopolysaccharide-stimulated macrophage-conditioned media (MCM+, 1:30 dilution) produced a reversible increase in an outward delayed rectifier K^+ current. **b** A summary graph illustrating the MCM+ significantly increases the outward delayed rectifier K^+ current in cultured hippocampal neurons and its blockade by a TEA ($n=10$). **c** Neurotoxic activity produced by the MCM+ in hippocampal neuronal cultures. TEA, a K_v channel antagonist, protected neurons against MCM-induced neurotoxicity. Neuronal injury was assessed after 24-h treatments with MCM+ by staining with a membrane-impermeable DNA-binding dye PI (Molecular Probes, Eugene, OR, USA) and counterstaining with a membrane-permeable DAPI. Note that MCM+ produced significant neuronal injury (in red) and TEA treatment blocked MCM-associated neuronal injury, suggesting the activation of K_v channels plays a crucial role in MCM-associated neuronal injury. **d** A summary data derived from **c** ($n=36$). The survival rates were calculated by counting the numbers of cells from the five different visual fields in each neuronal culture dish treated with DAPI (blue) and PI dyes (red).

* $p < 0.05$ vs. control, # $p < 0.05$ vs. MCM+