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Nerve Growth Factor Signaling Pathways Modulate HIV Vpr'sactions on Sensory Neurons: A Potential Target for Treatment of Distal Sensory Polyneuropathy in HIV/AIDS

Klaus Ballanyi[1], Christopher Power[2], Shaona Acharjee[3] and Christine A Webber[4]*

[1]*Department of Physiology, University of Alberta, Edmonton, Alberta, Canada*
[2]*Department of Medicine, University of Alberta, Edmonton, Alberta, Canada*
[3]*Department of Physiology and Pharmacology and Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada*
[4]*Department of Surgery, University of Alberta, Edmonton, Alberta, Canada*

Abstract

Over 35 million people are infected currently with the Human Immunodeficiency Virus (HIV), of whom 30-50% will experience Distal Sensory Polyneuropathy (DSP), usually causing paresthesiae and neuropathic pain, particularly in the feet. This presentation is identical to patients with Diabetic DSP. Current regimens for treating neuropathic pain have limited benefits. Thus, a deeper understanding of the mechanisms of HIV-DSP is imperative to permit the rational development of new therapies. Transgenic mice expressing the HIV-1 viral protein R (Vpr) show footpad epidermal denervation and allodynia as observed in HIV-infected patients. We found that exogenous Vpr inhibits axon outgrowth, causes hyperexcitability and increases cytosolic calcium in cultured dorsal root ganglion neurons (DRGN). Exposure of DRGN to nerve growth factor (NGF) or modulating NGF signaling pathways before Vpr treatment can block its effects. These findings will be extended to *in vivo* models to determine if altering the NGF signaling pathway can prevent Vpr-induced denervation and allodynia.

Keywords: Human Immunodeficiency Virus; Acquired Immunodeficiency Syndrome; Nerve Growth Factor; Dorsal root ganglion neurons; p75 neurotrophic receptor; Distal sensory polyneuropathy; Viral Protein R

Introduction

Distal sensory polyneuropathy (DSP) is the major peripheral nervous system disorder in 30-50% of people infected with Human Immunodeficiency Virus (HIV) showing Acquired Immunodeficiency Syndrome (AIDS). HIV-DSP symptoms include chronic neuropathic pain, allodynia, hyperalgesia, dysesthesia and gait dysfunction [1-10]. Current analgesics such as opioids, tricyclic antidepressants, anticonvulsants, capsaicin and topical anesthetics, show limited benefits as treatments for HIV-DSP and moreover, are often poorly tolerated [11-14]. Therefore, neuropathic pain associated with DSP can have devastating effects on the quality of life for affected patients, leading to depression and, at times, suicide. Importantly, current antiretroviral therapy regimes have little impact on HIV-DSP [8,15,16] and in fact older antiretroviral drugs (e.g. stavudine, zalcitabine, didanosine), actually worsened the signs and symptoms of DSP. HIV-1 encodes several accessory proteins including the 96 amino acid (14 kD) viral protein R (Vpr) [17], which is required for HIV infection of macrophages. Vpr expression in brain macrophages/microglia causes a neurodegenerative phenotype that resembles HIV-associated neurocognitive disorder ('Neuro-AIDS') [18]. The latter report indicated that impairment of CNS neurons is mediated by Vpr effects on ionconductance's, thus altering their membrane potential while it also appears to initiate apoptosis by promoting caspase-3 and -9 activation. Herein we review Vpr's involvement in DSP.

Possible sites and mechanisms of neuropathic pain in HIV-DSP

The lack of a targeted specific treatment of HIV-DSP is related to a limited understanding of both the primary anatomical site of injury and cellular mechanism(s) underlying DSP. The following comments are based on our recently published findings along with excellent reviews [5,6,10,19-21] as summarized in Figure 1. One of three candidate anatomical sites of injury is within the spinal cord where dorsal horn neurons might have developed long-term potentiation and/or disinhibition due to excessive or diminished synaptic inputs. Altered pathological inputs might originate from primary sensory dorsal root ganglion neuronal somas (DRGN) or their axons that might have been injured in the early stage of the infection. This central sensitization is transmitted to thalamic, reticular and then ultimately to cortical neural circuits to evoke pain sensation. A second candidate site lies in the extremities, particularly the feet where DSP-associated pain, numbness and paresthesia are typically perceived. Specifically, the site is within the skin of the feet at free nerve endings of distal axons ('fibers') that specifically sense pain ('noci'), mechano- or thermo-stimuli. This view is supported by data showing loss of epidermal free nerve endings in calf skin biopsies from affected patients with DSP [13]. Accordingly, chronic *versus* allodynic pain may be due to spontaneous action potential firing in injured distal axon endings of DRGN nociceptors versus mechano- or thermoreceptors, respectively. Finally, a third site of injury may be due to altered discharge within the DRGN soma located close to the spinal cord. These action potentials could propagate along the proximal ('central') axon to the dorsal horn via the same route as action potentials originating from the distal axon. It is therefore unknown whether such potential peripheral hyperexcitability in HIV-

***Corresponding author:** Christine A. Webber, Division of Anatomy, Department of Surgery, University of Alberta, Edmonton, Alberta, Room 501 Medical Sciences Building, T6G 2H7, Canada, Tel: 780-248-1886; E-mail: webber2@ualberta.ca

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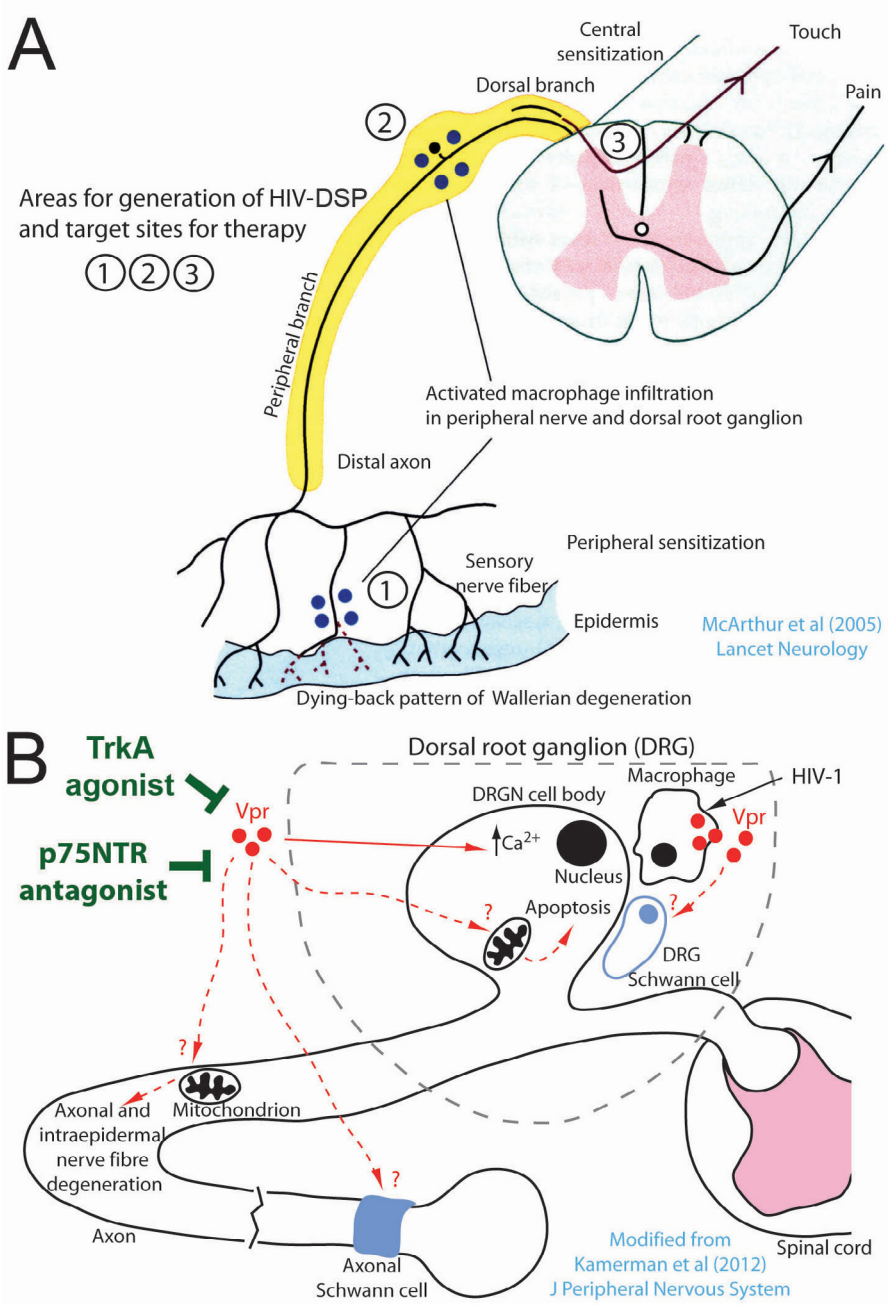

Figure 1: A, Three distinct sites of origin of HIV-related distal sensory polyneuropathy (HIV-DSP) are also the targets for a novel pharmaceutical therapy: (1) sensory nerve endings (distal axons) in skin (particularly feet), (2) dorsal root ganglion (DRG) with DRG neuron (DRGN) cell bodies of distal axons, (3) spinal cord and other central neural circuits, particularly in the somatosensory cortex. **B**, HIV-infected macrophages release viral coat protein (Vpr) that acts on DRGN/axons to mimic HIV-DSP symptoms, e.g. distal axon loss, allodynia and chronic pain. Potential targets of Vpr actions are cytosolic Ca^{2+} homeostasis, mitochondria or (Schwann cell) glial cells. We showed that p75 neurotrophin receptor ($p75^{ntr}$) antagonism blocked Vpr-induced axon inhibiting effects on DRGN.

DSP pain manifests at the DRGN soma and/or distal axon and which specific DRGN classes are involved.

As stated above, experimental models suggest that Vpr appears to be causally involved in HIV-related damage of CNS neurons [18]. This finding prompted our group to study Vprrolesinperipheral processes involved in HIV-DSP as summarized in Figure 2 and 3. Two animal models were instrumental for our findings in that regard. Firstly, we developed transgenic *vpr*/RAG1$^{-/-}$ mice which, similar to HIV/AIDS patients, constitutively express Vpr while being immunodeficient due to the absence of mature B or T cell lymphocytes [22]. Secondly, we used cultured DRGN from neonatal and adult rats (as well as human fetal DRGNs) to study the effects of exogenous (recombinant) Vpr under defined *in vitro* conditions [22,23]. We found that *vpr*/RAG1$^{-/-}$ mice display epidermal denervation and allodynia (also seen in the lower extremities HIV-infected people) but not control mice, while the animal's footpads show decreased expression of nerve growth factor

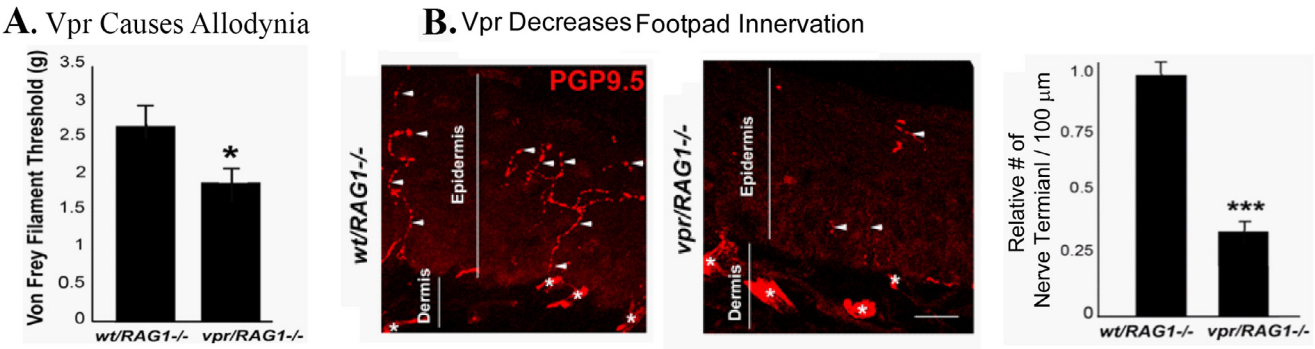

Figure 2: Our published findings established that *in vivo* Vpr expression mimics HIV-DSP features in transgenic immunosuppressed *vpr*/RAG1$^{-/-}$ mice [22,23]. A) Von Frey filament testing confirmed *vpr*/RAG1$^{-/-}$ mice have a lower pain threshold than their RAG1$^{-/-}$ age-matched control mice. B) Epidermal nerve fiber counts indicated the *vpr*/RAG1$^{-/-}$ mice have significantly less free nerve terminals than the RAG1$^{-/-}$ age-matched control mice.

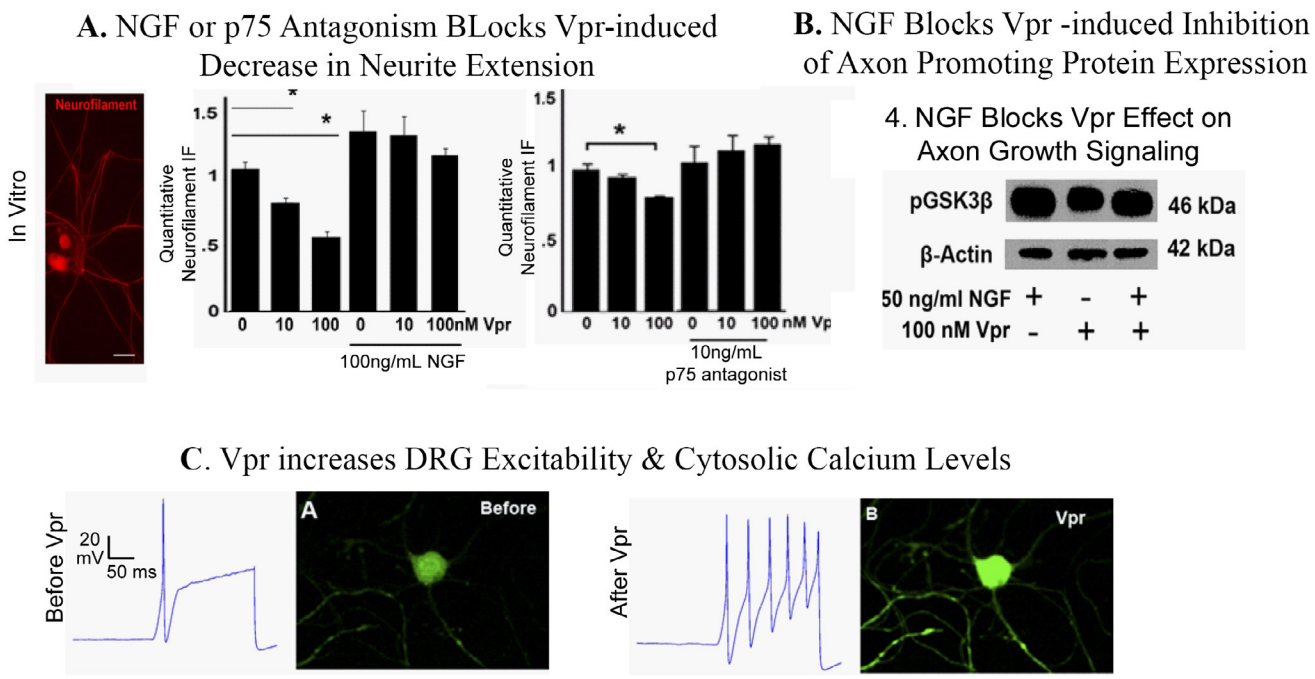

Figure 3: Our studies also showed and that nerve growth factor (NGF) counters Vpr's effects by acting on TrkAR and p75^{ntr} signaling. A) DRG neuronal cultures pre-treated with NGF or a p75 receptor antagonist were protected from the neurite-inhibiting effects of recombinant Vpr. B) p75 receptor antagonism blocks the Vpr-induced decrease in the neurite promoting protein pGSK3β. C) Vpr immediately causes an increase in intracellular calcium levels in DRG neurons which is inhibited by pretreatment with p75 receptor antagonism.

(NGF) [22,23]. It is interesting to note that HIV- and Diabetic DSP both display a denervation at the site of pain as well as a decreased NGF expression in the skin [23-25]. As it has been previously understood that NGF supports survival and skin innervation in adult animals and enhances regeneration of small diameter nociceptive DRGN [26-29], this indicated that NGF depletion might be involved in the pathogenesis of HIV-DSP. In support of this hypothesis, a clinical trial showed that NGF injection (0.1-0.3 µg/kg) into the feet of HIV/AIDS patients improved neuropathic pain symptoms [4]. However, the therapeutic potential of the approach was limited in that study by the occurrence of painful inflammation at the injection site. Based on these findings, we hypothesized that epidermal NGF depletion plays also a pivotal role in distal denervation of DRGN in our *in vivo* and *in vitro* animal models and thus studied the involvement of specific NGF receptors in Vpr-mediated HIV-DSP.

The noci- and mechanoreceptive DRGN peripheral axons that reside in the skin, a source of NGF, express two NGF receptors, the high-affinity tyrosine kinase-A receptor (TrkAR) and the low-affinity pan-specific p75 neurotrophin receptor(p75^{ntr}) [27,28]. Previous studies on cultured DRGN have shown that NGF (0.1-100 ng/ml) binds to the TrkAR to phosphorylate glycogen synthase kinase β (pGSK3β) and activate the phosphoinositol-3 kinase signaling cascade for promoting axon outgrowth [18,27]. In contrast, at higher doses (100-1000ng/ml), NGF binds to the p75^{ntr} to inhibit axon outgrowth in DRGN [30]. In

line with these observations, we showed in cultured rat DRGN that recombinant Vpr (100 nM) decreases expression of both TrkA receptor and pGSK3B which hampers neurite extension and increases their membrane excitability with a concomitant rise of cytosolic calcium [23]. Moreover, we demonstrated in this report that pre-exposure of DRGN to NGF (50 ng/ml) negates these Vpr-mediated effects, thus uncovering a potential target for a rational pharmacological HIV-DSP therapy. In that regard, we also found in that study that both blocking the $p75^{ntr}$ with the functional antibody REX and activating TrkAR *in vitro* with a TrkAR agonist inhibits Vpr-mediated inhibition of neurite growth similar to application of NGF.

These exciting findings suggest a possible molecular means by which Vpr affects TrkA/p75 signaling, however the mechanism is not yet know. It is possible that Vpr directly or indirectly binds to one or both the receptors, affecting their internalization and or their intracellular signaling. Our calcium imaging data, however clearly shows that NGF specifically inhibits the Vpr-induced increase in intracellular calcium, strongly suggesting a direct means for NGF to block the Vpr-induced affects *in vitro* [23]. Thus we do not believe that Vpr and NGF merely compete for pro-apoptotic and pro-survival pathways, respectively.

Where do we go from here?

The above study involving *in vitro* neurite growth, molecular signaling and Ca^{2+} imaging analyses indicated two potential pharmacological strategies for designing a specific treatment for HIV-DSP, i.e.by activating the TrkAR and/or inhibiting the $p75^{ntr}$ (or their associated signaling pathways). While systemic application of a successful novel drug may treat the disease, it is still important to identify the primary site of its action. Research on HIV-DSP using DRGN cultures, including our above studies of Vpr effects, has allowed for dose response curve determination of HIV-DSP-related neurotoxic agents and testing of candidate therapeutic drugs. Such tests were mostly based on patch-clamp recording from the DRGN somata or live-cell imaging to monitor how the drugs influence the axonal outgrowth peripheral sensory neurons and their viability evidenced e.g. via fluorescence imaging of cytosolic calcium or mitochondrial potential dynamics. Certainly, data from DRGN cultures are pivotal for the understanding of molecular signaling pathways in HIV-DSP and other types of neuropathic pain. However, cultures have also limitations regarding identifying the primary site from which pain in HIV-DSP originates. Specifically:*(i)* modality-specific sensitivity of affected DRGN somata and/or axons, as in the intact animal, cannot be determined *in vitro*, (*ii*) ion channel dysfunction in cultured DRGN, mostly obtained from fetal or neonatal animals, may differ from that in adult cells *in vivo*, (*iii*) culture conditions may affect ion channel expression, (*iv*) cellular processes cannot be identified as dendrites *versus* proximal or distal axons and, consequently (*v*) patch-clamp recording does not delineate if affected ion channels are located on the DRGN soma, dendrites or (distal) axon.

To overcome these limitations, we propose to focus future efforts on identifying in *vpr*/$RAG1^{-/-}$ mice [22] both the primary site and ionic mechanism in the proposed DRGN/axon hyperexcitability. Specific affected DRGN classes can be identified either in anesthetized or decerebrated *vpr*/$RAG1^{-/-}$mice *in vivo* via adequate footpad sensory stimulation combined with recording of their extracellular action potential discharge. For these studies, we will perform a laminectomy of the lumbarspinal cord to enable compound or single fiber action potential recording with hook electrodes or, alternatively, with fine tungsten electrodes for microneurography of the DRG axons [6,31-35]. We expect that such analyses will reveal that nociceptive DRGN/axons are spontaneously active and theiractivation threshold is lower in *vpr*/$RAG1^{-/-}$ mice compared tocontrol mice. Moreover, allodynia in *vpr*/$RAG1^{-/-}$ mice might be related to ectopic discharge in (normally 'silent') C fibers and possibly also in mechanosensitive Aβ or thinly myelinated A™pain fibers. If such axons are spontaneously active in*vpr*/$RAG1^{-/-}$ mice, this might be indicative of paresthesia [36]. It is also possible that the action potential threshold in thermosensitive axons is changed in *vpr*/$RAG1^{-/-}$ mice and that they show spontaneous and/or ectopic firing. Underlying (ion channel) dysfunctions can be studied using either intracellular microelectrode or patch-clamp recording from DRGN somata in the functionally intact preparations or by microneurography and threshold tracking in the distal axons [6,31,33-35,37].

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