Journal of NeuroVirology, 7: 183–185, 2001
 ISSN 1355–0284/01 $12.00+.00

Letter to the Editor

Effect of extracellular HIV-1 Vpr protein in vitro

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We have noted the article by Huang *et al* (2000) with great interest and are pleased to see our previous findings confirmed in another laboratory. We read with interest that extracellular Vpr does not cause G2/M arrest at least in C6 glioma cells, confirming our data that it localizes at the cell membrane. However, there are several points that are unclear.

The statement that neither Annexin V nor TUNEL staining are enough to discriminate between necrosis and apoptosis is perplexing as these are the most widely used assays in the apoptosis field. Seeing that Annexin V, TUNEL, and DNA fragmentation pointed towards apoptosis, it is not apparent why Huang *et al* claim the effects of Vpr to be more necrotic than apoptotic in the abstract.

In contrast to Huang *et al*'s (2000) interpretation of our statement in Piller *et al* (1998), we would like to clarify that our previous results with glioma cells in primary rat cultures indicated that glioma cells were *more* susceptible to Vpr effects than hippocampal neurons (see Figure 1). This finding is in agreement with the results of Huang *et al* (2000) and does not conflict with our theory that Vpr's membrane insertion and ion channel function are dependent on membrane potential as glioma cells are known to maintain more negative resting membrane potentials (−70 to −90 mV) than neurons.

Although Huang *et al* (2000) state not to have seen any effects of Vpr on cortical neurons, they report about a 50% decline in number of cortical neurons after Vpr treatment. Interestingly, the time point in these experiments was much sooner after Vpr addition than in any other experiment. This does not change their main observation that Vpr affects even similar cell types differently, and we agree that the exact reason for this needs to be further investigated.

We would like to suggest that our analysis of the different regions of Vpr may shed some light on the differential effects of Vpr (Piller *et al*, 1999). The C-terminus of Vpr appeared to be cytotoxic (to both neurons and glioma) without causing membrane depolarization whereas the N-terminal 40 amino acids of Vpr caused cytotoxic effects, cell depolarization, and ion channels. Therefore, at least two different mechanisms of action seem to be

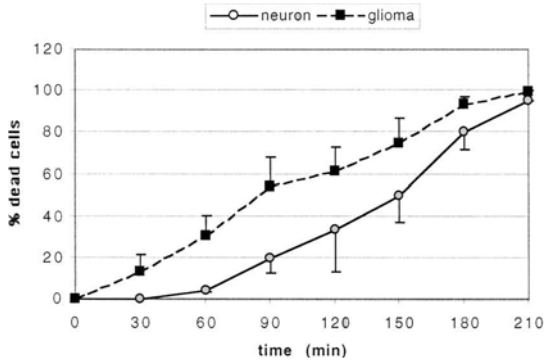

Figure 1 Cytotoxic effect of extracellular, purified, recombinant Vpr expressed as a percentage of dead cells. Data are the mean of 3–7 experiments and error bars are standard errors.

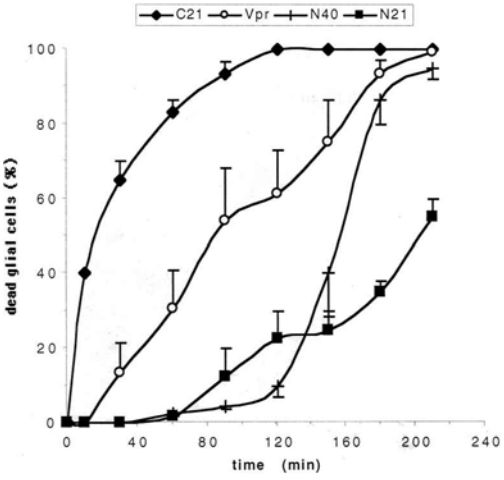

Figure 2 Cytotoxic effects of extracellular addition of WT Vpr and Vpr peptides (C21, N21, N40) on glial cells expressed as percentage of dead cells.

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responsible for Vpr-induced cytotoxic effects, and only the ion channel-causing pathway was found to depend on the resting membrane potential in our previous work. The mechanism of the cytotoxic activity of the C-terminal portion of Vpr seems to be different, and our data suggest that glioma cells may be even more affected by the C-terminal Vpr cytotoxicity (Figure 2) than hippocampal neurons (see Figure 8 in Piller *et al*, 1999). Therefore, in addition to the membrane potential requiring cytotoxic effect of Vpr, a second cytotoxic mechanism of the C-terminus appears to be more important in some cells. A wider range of different cell types needs to be screened for both the N-terminal and C-terminal cytotoxic effects to test this hypothesis.

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Response

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When identifying apoptosis, several assays, including the definitive morphological assay and fragmentation assay, should be used to avoid confusing apoptosis with necrosis (Grasl-Kraupp *et al*, 1995). Thus, although the data from our assays (Huang *et al*, 2000) suggest apoptosis, that from the definitive morphology and fragmentation assays suggested both apoptosis and necrosis were occurring in these cultures. We also would like to thank the author for providing new data clarifying their statement in Piller *et al* (1998). This evidence clearly shows that astrocytes are affected by the Vpr protein, causing cytotoxicity. In response to their statement concerning loss of cortical neurons, we were never able to identify apoptotic neurons in Vpr-treated cortical cultures as assayed by screening for NSE/TUNEL double-labeling (Grasl-Kraupp *et al*, 1995). We collected a body of unpublished data, including images from Vpr-treated cultures exposed 24 h or more. We never observed cytotoxic cortical neurons as we did in the hippocampal cultures. This is also true for peripheral neurons (rat superior cervical ganglion neurons) which we have harvested and maintained in relatively pure culture. Additionally, based on the results presented in the author's letter, and assuming a similar cytotoxicity mechanism in hippocampal and cortical neurons, neuronal cytotoxicity in our cortical cultures should have (but did not) occurred early, 90 min, and peaked at 4 h (Grasl-Kraupp *et al*, 1995). Finally, the author's data in this letter and previous publications suggest a fast C-terminal cytotoxic mechanism that acts differentially on astrocytes (e.g., a membrane receptor not expressed in neurons), and a slower N-terminal mechanism that acts on both astrocytes and neurons (Piller *et al*, 1999).

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