Ac-YVAD-2,6-DIMETHYLBENZOYLOXY-METHYLKETONE (Ac-YVAD-DMK) MINIMIZES CYTOCHROME C TRANSLOCATION BUT NOT IL-1β INCREASE BY HIV-1 GP120 IN THE NEOCORTEX OF RAT

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Intracerebroventricular (i.c.v.) injection of gp120 in the adult rat causes microglial cell activation and enhanced expression of IL-1β and this seems to be responsible for neuronal apoptosis in the neocortex (see Corasaniti *et al.*, 2001). Thus, the antagonist of IL-1 receptor type I (IL-1ra) and Ac-YVAD-chloro-methylketone (Ac-YVAD-cmk), a specific inhibitor of caspase 1, afford neuroprotection (see Corasaniti *et al.*, 2001). Here we now report that gp120 induces cyt-c translocation and this, but not elevated IL-1β, is minimized by Ac-YVAD-dmk, a caspase-1 inhibitor (Thornberry *et al.*, 1992).

Under chloral hydrate (400 mg/kg i.p.) anaesthesia, male Wistar rats (250-280 g) were stereotaxically implanted with a guide cannula (25 gauge) into one lateral cerebral ventricle as previously described (see Bagetta et al., 1999). The animals were allowed 4 days recovery before treatment. Then, a single dose (100 ng i.c.v.; 2 μl volume; 1μlmin^{-1} rate) of gp120 was given alone or in combination with caspase 1 inhibitors to each rat with a 5 μl Hamilton syringe. Six hours after the last treatment the animals have been sacrificed and the brain cortical tissue dissected out. Immunoreactive IL-1β was assayed in cytosolic and mitocondrial fractions from individual brain cortical tissue samples by an established, rat specific ELISA (see Corasaniti *et al*., 2001); cyt-c (mouse monoclonal ab Pharmingen San Diego, CA; 1:2000 dilution) was measured by western blotting (see Corasaniti *et al.*, 2001). Cyt-c has also been studied by immunogold electron microscopy (Pharmingen San Diego, CA; 1:10 dilution). In the antagonism study, injection of gp120 was preceded (1 h beforehand) by Ac-YVAD-dmk or by Ac-YVAD-cmk. Immunoelectron microscopy (n=3) and western blot analysis (n=3) demonstrate that gp120 causes cyt-c translocation in the brain neocortex of rat. Densitometric analysis (see Corasaniti *et al.*, 2001) of cyt-c immunoreactive band revealed that Ac-YVAD-dmk (50 pmoles i.c.v.) caused an approx. 60% reduction of cyt-c vs gp120 (103.3±8.2 vs 160.3±16.4 arbitrary units; $P<0.05$, ANOVA followed by Tukey-Kramer). This treatment did not affect gp120 increased mitochondrial (10.7±2.4 vs gp120 9.4±1 pmolmg^{-1} protein; $P>0.05$) and cytosolic (12.3±4.1 vs gp120 9.1±1.2 pmolmg^{-1} protein) IL-1β. Likewise Ac-YVAD-dmk, a neuroprotective dose (100 pmoles i.c.v.) of Ac-YVAD-cmk (Bagetta *et al*., 1999), did not reduce enhanced IL-1β levels by gp120 into the cytosolic (11.7±3.1 pgmg^{-1} protein; n=3, $P<0.05$ vs control) and mitochondrial (21.3±1.7 pgmg^{-1} protein; n=3, $P<0.001$ vs control and vs gp120 given alone) fractions. In conclusion, under the present experimental conditions, caspase 1 inhibitors seem to afford neuroprotection via inhibition of cyt-c translocation.

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Bagetta G. *et al.* (1999) Neuroscience 89, 1051-1066. Corasaniti M.T. *et al.* (2001) J. Neurochem. 79, 1-8. Thornberry N.A. *et al.* (1992) *Nature,* 356**,** 768-774.