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Glutamate and purine catabolites in relation to free radical production during focal ischemia-reperfusion: an *in vivo* study in cats

M. Valentino, R. Graf, S. Toyota, C. Dohmen, P. Gabel, W.-D. Heiss

Max-Planck-Institute for Neurological Research, Cologne, Germany
E-mail: Mario.Valentino@pet.mpin-koeln.mpg.de

Introduction: The *in vivo* interrelation between excitotoxicity and oxidative stress following cerebral ischemia in cats was investigated. To elucidate the role of this mechanism in cerebral ischemia, the study presented herein sought to investigate the spatial and temporal features of the free radical response to elevations of glutamate and purine catabolites in a reproducible model of *in vivo* focal ischemia/reperfusion. The time course of neurochemical and Reactive Oxygen Species (ROS) were simultaneously conducted in ischemic focus and perifocal region of the brain.

Methods: Prolonged middle cerebral artery occlusion (MCAO) was induced in halothane anesthetized cats (n=6) for a 3 hr period followed by 6 hr of reperfusion. Microdialysis probes (membrane length 1.0mm, diameter 0.5mm) were stereotactically implanted in ischemic focus and perifocal region of the cortex; the probes were perfused with aCSF containing 2mM salicylate at a rate of 2μL/min and the dialysate (30 min fractions) were analysed by HPLC for the hydroxylated adducts 2,3-and 2,5-dihydroxybenzoic acids (DHBA), amino acids and purine catabolites. Laser Doppler probes were placed in close proximity to the microdialysis probes to measure regional CBF in both gyri. Deep body temperature, brain temperature, mean arterial blood pressure and arterial blood gases were controlled throughout the course of the experiments.

Results: MCAO reduced regional CBF in all animals below 25% of control in the ischemic focus and at around 45% in the perifocal region of the brain. Extracellular glutamate, hypoxanthine and xanthine increased by 8, 2 and 0.2 fold above basal levels in the ischemic focus whereas hypoxanthine increased by 1 fold, with glutamate and xanthine staying close to basal levels in the perifocal region. Recovery to basal levels of glutamate, hypoxanthine and xanthine extended well into the reperfusion phase in the ischemic focus compared to the fast recovery of hypoxanthine and xanthine in the perifocal region. Salicylate hydroxylation was increased substantially in the perifocal region compared with samples in the ischemic focus. An increase in 2,3-DHBA within the perifocal region was observed during ischemia, and a second progressive increase was observed during reperfusion. In contrast, there was no significant increase in 2,3-DHBA within the ischemic focus throughout the ischemic phase, and a delayed lower increase during reperfusion.

Conclusion: As the glutamate rise is predominantly intraischemic whereas ROS is mainly post-ischemic, these results favour the view that glutamatergic mechanisms may be a trigger for the Ca-activated proteolytic conversion of xanthine dehydrogenase to its oxidase. With reperfusion, xanthine oxidase catalyses the conversion of hypoxanthine to urate forming superoxide and peroxide and eventually $OH^{\cdot}$. Our experiments show that since substrate availability for xanthine oxidase is by far greater in the ischemic focus than in the perifocal region, but it is in the perifocal region that the greater part of $OH^{\cdot}$ is produced, than it is apparent that the xanthine-xanthine oxidase pathway is an unlikely source of ROS in reperfusion injury. These findings are consistent with the concept that the role of ROS in cerebral injury may vary qualitatively and or quantitatively in areas of total or partial cerebral perfusion.