

## EVIDENCE TO IMPLICATE THE ENDOCANNABINOID SYSTEM IN RETINAL DAMAGE CAUSED BY HIGH INTRAOCULAR PRESSURE (IOP)-INDUCED ISCHEMIA IN RAT

Luigi A. Morrone<sup>1</sup>, Carlo Nucci<sup>2,3</sup>, Laura Rombolà<sup>1</sup>, Federica Cavaliere<sup>1</sup>, Angelica Cerulli<sup>2</sup>, Mauro Maccarrone<sup>4</sup>, M. Tiziana Corasaniti<sup>3,5</sup>, Giacinto Bagetta<sup>1</sup>

<sup>1</sup>Dept. of Pharmacobiol., Univ. of Calabria, Cosenza; <sup>2</sup>Dept. of Biopathology and Diagnostic Imaging, Univ. Tor Vergata of Rome; <sup>3</sup>IRCCS C. Mondino Foundation, Mondino-Tor Vergata Center for Exp. Neuropharmacol., Rome; <sup>4</sup>Dept. of Biomedical Sciences, Univ. of Teramo; <sup>5</sup>Dept. of Pharmacobiological Sciences, Univ. Magna Graecia, Catanzaro, Italy

A number of studies have shown that synthetic cannabinoids exert neuroprotective actions in the eye with potential implications for the treatment of glaucoma (1). The present study was carried out to evaluate whether modifications of the endocannabinoid system may indeed be associated with retinal damage caused by ischemia induced by high IOP (2). Anandamide (AEA) synthesis, transport, hydrolysis and AEA endogenous levels were assessed by means of HPLC in the retina of rats undergoing 45 min ischemia followed by 12 h reperfusion. Under these experimental conditions, binding to cannabinoid (CB1R) and vanilloid (TRPV1) receptor was assessed using rapid filtration assays. AEA-hydrolase (fatty acid amide hydrolase, FAAH), CB1R and TRPV1 protein content was determined by ELISA. To characterize the neuroprotective profile of drugs that interfere with the endocannabinoid system, cell counting in the retinal ganglion cell (RGC) layer and RT-PCR for Thy-1 mRNA expression were used. In rat retina, ischemic insult followed by reperfusion results in enhanced FAAH activity and protein expression paralleled by a significant decrease of the endogenous AEA tone whereas the AEA-membrane transporter or the AEA-synthase NAPE-PLD (N-acylphosphatidylethanolamines-hydrolyzing-phospholipase D) were not affected. Retinal ischemia/reperfusion decreased the expression of cannabinoid (CB1) and vanilloid (TRPV1) receptors. Systemic administration of the specific FAAH inhibitor, e.g. URB597, reduced enzyme activity and minimized retinal damage. Similarly, intravitreal injection of the AEA stable analogue, R(+)-methanandamide, reduced cell loss in the RGC layer and this was prevented by systemic administration of a CB1 or TRPV1 selective antagonists, e.g. SR141716 and capsazepine, respectively.

In conclusion, the original observation that retinal ischemia/reperfusion reduces endogenous AEA via enhanced expression of FAAH supports the deduction that this is implicated in retinal cell loss caused by high IOP in the RGC layer.

Partial financial support by the Ministry of Health (RF 2005) is gratefully acknowledged.

- 1) Tomida I., Pertwee R.G. and Azuara-Blanco A. (2004) Br. J.Ophthalmol. 88:708-713.
- 2) Osborne N.N., Casson R.J., Wood J.P.M., Childlow G., Graham M. and Melena J. (2004) Prog. Ret. Eye Res. 23: 91-147.