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MPEP protection against hepatic ischemia/reperfusion injury is associated to TNF-α decrease

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The Negative Allosteric Modulator (NAM) 2-methyl-6-(phenylethynyl) pyridine (MPEP) of metabotropic Glutamate Receptor subtype 5 (mGluR5) improves viability of isolated anoxic hepatocytes1. It is known that during ischemia/reperfusion (I/R) injury, Kupffer cells activation occurs, causing an increase in TNF-α production. Moreover, TNF-α positively correlates with iNOS expression through NF-κB[2,3] and because of the presence of TNF Response Element on iNOS gene4,5.The aims of this study were: to investigate the protection mediated by other NAMs (MTEP and Fenoabam) against anoxic damage in isolated hepatocytes and to investigate the liver functionality in two *ex vivo* models of I/R. Male Wistar rat hepatocytes were exposed to 90 minutes anoxia at 37°C with: MPEP and 3-((2-methyl-4-thiazolyl ethynyl pyridine (MTEP) at 3-30μM, Fenobam at 1-10-50-100μM. Hepatocytes viability was evaluated by trypan blue exclusion and LDH release. Wildtype and mGluR5 knockout livers from Balb-c mice were isolated, subjected to cold or normothermic I/R and treated with MPEP 0.3μM; transaminases release, BAX and Bcl-2, iNOS, eNOS and TNF-α protein expression were evaluated in cold I/R samples, while LDH, AST, TNF-α release were evaluated in normothermic samples. MPEP 30μM, MTEP 3μM and Fenobam 50μM improved significantly anoxic isolated hepatocytes viability respect to anoxic controls. MPEP addition during I/R significantly reduced LDH, transaminases and TNF-alpha respect to ischemic controls in both cold and normothermic I/R, with a trend similar to mGluR5 knockout samples. Furthermore, MPEP reduced TNF-α levels, causing selectively iNOS expression decrease without affecting eNOS expression. Our results demonstrated that MPEP, MTEP and Fenobam protect rat hepatocytes against ischemic injury. Furthermore, MPEP is able to reduce susceptibility of liver grafts to the preservation injury in two *ex vivo* models, in which the pharmacological blockade of mGluR5, interrupting the excitotoxic cascade, reduces the inflammation mediator TNF-α.

Recent Publications

1. Storto et. 2000. Selective blockade of mGlu5 metabotropic glutamate receptors protects rat hepatocytes against hypoxic damage. *Hepatology.*
2. Nandi et al. 2010. TNF-α modulates iNOS expression in an experimental rat model of indomethacin-induced jejunoileitis. *Molecular and Cellular Biochemistry*, 336(1–2), pp.17–24
3. Fonseca et al. 2003. TNF-α mediates the induction of nitric oxide synthase in macrophages but not in neutrophils in experimental cutaneous leishmaniasis. European Journal of Immunology, 33(8), pp.2297–2306
4. Muntané et al. 2000. TNF-alpha dependent production of inducible nitric oxide is involved in PGE(1) protection against acute liver injury. *Gut,* 47(4), pp.553–62
5. Medeiros et al. 2007. Connecting TNF- α Signaling Pathways to iNOS Expression in a Mouse Model of Alzheimer's Disease: Relevance for the Behavioral and Synaptic Deficits Induced by Amyloid Protein. *Journal of Neuroscience*, 27(20), pp.5394–5404.

Biography

Clarissa Berardo, Doctorate in Biomedical Sciences, curriculum Pharmacology. Master Degree in *Molecular Biology and Genetics.* She has her expertise in mouse and rat liver isolation to study preservation techniques for organ transplantation (Cold Storage and Machine Perfusion), hepatocytes isolation from mouse and rat, primary and tumoral (HepG2 and Huh7.5) cell culture, use of genetic (Zucker) and nutritional (Methionine and Choline Deficient Diet) animal models to study hepatic diseases, such as NAFLD, NASH and ischemia/reperfusion injury. Knowledge of principal techniques performed in biomedical laboratory: western blot analysis, RT-PCR, histological techniques, biochemical and enzymatic assays.

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