Multidrug Resistance-Associated Protein 4 Expression in Ammonia-Treated Cultured Rat Astrocytes and Cerebral Cortex of Cirrhotic Patients with Hepatic Encephalopathy

Abstract:
Hepatic encephalopathy (HE) is a neuropsychiatric syndrome frequently accompanying liver cirrhosis and reflects the clinical manifestation of a low grade cerebral edema associated with cerebral oxidative/nitrosative stress. The multidrug resistance-associated protein (Mrp) 4 is an export pump which transports metabolites that were recently suggested to play a major role in the pathogenesis of HE such as neurosteroids and cyclic nucleotides. We therefore studied Mrp4 expression changes in ammonia-exposed cultured astrocytes and postmortem human brain samples of cirrhotic patients with HE. NH4Cl increased Mrp4 mRNA and protein levels in astrocytes in a dose- and time-dependent manner up to threefold after 72 h of exposure and concurrently inhibited N-glycosylation of Mrp4 protein. Upregulation of Mrp4 mRNA and protein as well as impaired N-glycosylation of Mrp4 protein by ammonia were sensitive towards the glutamine-synthetase inhibitor l-methionine-S-sulfoximine and were not induced by CH3NH3Cl (5 mmol/L). Upregulation of Mrp4 mRNA required ammonia-induced activation of nitric oxide synthases or NADPH oxidase and p38(MAPK)-dependent activation of PPAR alpha. Inhibition of Mrp4 by ceefourin 1 synergistically enhanced both, inhibition of astrocyte...
proliferation as well as transcription of the oxidative stress surrogate marker heme oxygenase 1 by forskolin (10 μmol/L, 72 h) or NH4Cl (5 mmol/L, 72 h) in cultured rat astrocytes. Increased Mrp4 mRNA and protein levels were also found in postmortem brain samples from patients with liver cirrhosis with HE but not in those without HE. The data show that Mrp4 is upregulated in HE, which may be relevant for the handling of neurosteroids and cyclic nucleotides in response to ammonia.