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STRES OKSYDACYJNY I NITRACYJNY WYWOŁANY PRZEZ JONY AMONOWE W ASTROCYTACH I KOMÓRKACH ŚRÓDBŁONKA NACZYŃ MÓZGOWYCH: PRÓBY PROTEKCJI

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Excessive accumulation of ammonia in the brain is a main causative factor in hepatic encephalopathy (HE), a neurological and neuropsychiatric syndrome associated with inefficient detoxification of ammonia in the liver. High concentrations of ammonia in the brain, resulting from acute liver failure, are well correlated with its elevated concentration in the blood. Brain edema related to astrocytic swelling is the most serious complicating factor of HE and major cause of death in patients with acute liver failure. According to many studies and observations, ammonia-induced brain edema is mainly cytotoxic in its nature, resulting from swelling of astrocytes, which are most exposed to detrimental effect of ammonia. Ammonia causes astrocytic swelling by triggering a vicious cycle of oxidative/ nitrosative stress (ONS) and hypotonic stress, concomitant with an array of metabolic disturbances.

One recently highly disputed and controversial issue is the existence of a vasogenic component of HE- induced CE. It has been observed that progression of intracranial pressure in the course of ALF is strictly correlated with the increase in blood brain barrier (BBB) permeability. Specifically, these ALF-related changes have been linked to events secondary to tight junction (TJ) protein degradation mediated by MMP-9. In astrocytes and neurons, ammonia-induced ONS leads to the oxidation and/or nitration of proteins and nucleic acids, and in this way directly modifies functions and metabolism of these cells. It may be speculated that ammonia by inducing ONS can also change the physiological properties of BBB–forming cells and in this way influence BBB permeability and alter brain homeostasis. Here, we tested the hypothesis that ammonia induces changes in blood–brain barrier (BBB) permeability by a mechanism coupled to ONS evoked in the BBB-forming cerebral capillary endothelial cells. We also tested the ability of a number of cytoprotective compounds to counter the effects of ammonia, both in astrocytes and brain capillary endothelial cells.

According to astrocytes, the hypothesis assuming protective effects of natriuretic peptides (NPs) was tested. We envisaged the possibility that NPs may attenuate ammonia-

induced ROS formation and that this may involve interaction with NPR-C. Signaling pathways associated with antioxidative properties of NPs and their cell protective actions have been subject of analysis.

We report that ANP and CNP reduces ROS formation both in ammonia-treated and non-treated astrocytes. The ROS reducing effect also occurred upon incubation with the NPRC- interacting ANP analog, $cANP_{(4–23).}$ Furthermore, the superoxide anion was the major ROS species accumulating following ammonia treatment and its accumulation was reduced both by ANP and $cANP_{(4–23).}$ The study documented the presence of a functional NPR-C receptor on astrocytes, the stimulation of which counteracts oxidative stress in these cells. Moreover, $cANP_{(4–23)}$ attenuated, by an as yet unknown mechanism, ammonia-induced accumulation of NO, and could in this way reduce nitrosative stress. Stimulation of NPR-C inhibited ammonia–induced astrocytes swelling, manifesting its potential as an edema-preventing agent.

Treatment of a rat brain endothelial cell line with ammonia caused accumulation of ONS markers: ROS, NO and products of lipid peroxidation, F_2-isoprostanes. One possible explanation for excessive RNS production is the fact that ammonia increases the expression of y^+LAT2, a transporter that mediates the uptake to the cells of the nitric oxide precursor, arginine. At the same time, ammonia increased the activity of extracellular matrix metalloproteinases (MMP-2/MMP-9) and increased cell permeability to fluorescein isothiocyanate-dextran (40 kDa), The increase of cell permeability was ameliorated upon co-treatment with a MMP inhibitor, SB-3CT and with an antioxidant, glutathione diethyl ester, which also reduced F_2-isoprostanes. Ammonia-induced ONS was attenuated by a cytoprotective agents l-ornithine, phenylbutyrate, and their conjugate l-ornithine phenylbutyrate (OP), an ammonia-trapping drug used to treat hyperammonemia. This shows that apart from its known peripheral protective effect, OP also possess direct cytoprotective

effects in endothelial cells, most likely related to the attenuation of biochemical manifestations of ONS.

The results support the concept that ONS and ONS-related activation of MMPs in cerebral capillary endothelial cells contribute to the alterations in BBB permeability and to the vasogenic component of cerebral edema associated with acute liver failure.

In conclusion, the results confirm that oxidative/nitrosative stress induced directly by ammonia in astrocytes and in the cerebral capillary endothelial cells is the trigger for events leading to both cytotoxic and vasogenic CE. Therefore, agents reducing ONS, such as the ones examined in the present study ($cANP_{(4-23)}$; OP) are good candidates for application in future therapies aimed at counteracting the detrimental effects of ammonia, first in animals with experimentally induced hyperammonemia , and eventually in patients with HE.