FUNCTIONAL IMPLICATION OF TRANSPORTERS DURING NITROSATIVE STRESS

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Nitrosative stress can be defined as the pathological state that involves a massive formation of nitrogen oxides (NOx); Examples of the pathological condition include inflammation, diabetes and neurodegenerative diseases. Because of the highly reactive nature of the oxide forms, the nitrosative stress is often associated with chemical modification of variety of DNA, proteins and lipids, which would ultimately lead to the alteration in the physiological function of target biomolecules such as transporters. In this presentation, two lines of investigations will be presented for alterations in functional activities of carriers expressed in barriers of blood/cerebrospinal fluid (CSF) and blood/brain during disease states with nitrosative stress. In the first example, the mechanism responsible for the reduced clearance of benzylpenicillin (BPC) from the CSF was investigated in rats that received an intracisternal administration of lipopolysaccharide (LPS). BPC was intravenously injected and its elimination from the CSF studied. During the inflammation created by the LPS administration to the cisterna magna, the clearance of BPC and taurine from the CSF was significantly reduced but reverted to the control level when N-nitro-L-arginine, a nitric oxide (NO) synthase inhibitor was intracisternally administered. The in vitro uptake of BPC and taurine was significantly reduced in the choroid plexus (CP, the blood-CSF barrier) of rats with experimental inflammation and in control CP that had been pretreated with sodium nitroprusside (SN, an NOx donor). Interestingly, the clearance and CP uptake of formycin B, a substrate for a nucleoside transporter, were not affected by the experimental inflammation or by pretreatment with SN. These observations suggest that the BPC transporter, and probably other transport systems as well, is functionally sensitive to NOx in the blood-CSF barrier. In the second example, functional outcome of P-glycoprotein (Pgp) during experimental diabetes will be presented. The kinetics of penetration of cyclosporine A, a well-known substrate for the transporter, across the blood-brain barrier (BBB) was determined in streptozotocin (STZ) induced diabetic rats, and compared with that in control rats. The brain uptake clearance of cyclosporine A in hyperglycemic rats was significantly lower than that in control rats as early as one week of STZ treatment and remained thereafter. Since the diabetic condition was associated with the enhanced level of NOx in the systemic circulation (i.e., the nitrosative stress), we were interested whether the pathological condition is related to the functional induction of the efflux transporter in the brain. Thus, MBEC4 cells, an in vitro model of the BBB, was used to study the potential involvement. The function of the efflux transporter was stimulated in the presence of NOx donors such as 3-morphinoisodionimine (SIN-1) and SN. The functional induction was associated with an elevated level of mRNA for Pgp during the nitrosative condition. Interestingly, however, the functional induction for Pgp was not apparent for certain nitric oxide (NO) donors such as S-nitroso N-acetylpenicillamine (SNAP) and diethylenetriamine (DETA), indicating that NO itself does not directly lead to the functional induction. Consistent with this hypothesis, the addition of ascorbate, an antioxidant, or superoxide dismutase, a scavenger of superoxide, partially reversed the functional induction by SN pretreatment in ME4 cells. These observations indicate that the conversion of NO to peroxynitrite may be related to the induced function of the efflux transporter in MBEC4 cells. Since the experimental diabetic rats showed the elevated level of NOx in the blood, the peroxynitrite-mediated induction of Pgp may occur for rats with experimental diabetes. Taken together, since the functional outcome of transporters is complicated (i.e., functional impairment, no effect or induction) during the nitrosative stress, the implication has to be carefully studied for the optimal pharmacotherapy.